Phase I Trial Evaluating Safety and Tolerability of the Irreversible Epidermal Growth Factor Receptor Inhibitor Afatinib (BIBW 2992) in Combination with the SRC Kinase Inhibitor Dasatinib for Patients with Non-small Cell Lung Cancer (NSCLC)

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Additional study sites within LCMC consortium may be reviewed for approval for PHASE IB EXTENSION portion of trial.

TABLE OF CONTENTS

TITLE PAGE **1**

TABLE OF CONTENTS **3**

PROTOCOL SYNOPSIS **7**

Figure 1: Flow chart of design 7

STUDY CALENDAR – PHASE IA **9**

STUDY CALENDAR – PHASE IB **10**

1 INTRODUCTION **11**

1.1 SIGNIFICANCE 11
1.1.1 Scope of the Problem: Treatment of NSCLC *11*
1.1.2 Epidermal Growth Factor Receptor (EGFR) Inhibitors in Lung Cancer *11*
1.1.3 EGFR: A Driver Mutation in NSCLC *12*
1.1.4 T790M Gate-keeper EGFR Mutation and Irreversible EGFR-TKI in NSCLC *12*
1.1.5 Mechanisms of EGFR-TKI Resistance in NSCLC *13*
1.1.6 Significance of the Src-TKI Dasatinib in NSCLC *13*
Table 1: Drugs that may overcome EGFR resistance. Adapted from [92] 14
1.1.7 SRC Signaling in Non-Small Cell Lung Cancer *14*
Figure 2. EGFR-mutant NSCLC in vitro results from Haura lab 16
1.2 COMBINATION OF AFATINIB WITH DASATINIB 16
1.3 CANCER RADIOMICS 17
1.3.1 Scope of Imaging Problem *17*
Table 1.3: Potentially Representative CT features and Variables. 18
1.3.2 Radiomics: A Rationale Solution *19*

2 DASATINIB DRUG PROFILE **21**

2.1 ACTIVITY IN EXPERIMENTAL MODELS 21
2.1.1 In Vitro Molecular Studies *21*
2.1.2 Lung Cancer Cell Studies *22*
2.1.3 In Vivo Studies *22*
Figure 3. Haura lab mutated NSCLC mouse model: synergistic activity 23
2.1.4 Preclinical Toxicology *23*
2.2 CLINICAL PHARMACOKINETICS 24
2.2.1 Absorption *24*
2.2.2 Distribution *24*
2.2.3 Metabolism *24*
2.2.4 Elimination *25*
2.2.5 Clinical Experience with Dasatinib in CML and Ph+ ALL *25*
2.3 SAFETY OF DASATINIB IN CLINICAL STUDIES 26
2.3.1 Safety in Hematologic Malignancy *26*
Table 2: Very Common and Common AEs Reported in Subjects in Clinical Studies 26
2.3.2 Experience in Phase 2 Breast Cancer Studies *30*
Table 3: Drug-Related Adverse Events in ≥ 25% Subjects for Phase 2 Breast Cancer Studies CA180059 and CA180088 30
2.3.3 Experience in Phase II Prostate cancer studies *30*
Laboratory Abnormalities *31*
Table 4: CTC Grades 3/4 Laboratory Abnormalities in CML and Ph+ ALL 31
2.4 ANTICIPATED ADVERSE EVENTS 32

2.4.1 Myelosuppression *32*
2.4.2 Bleeding Related Events *32*
2.4.3 Fluid Retention *32*
2.4.4 QT Prolongation *33*
2.4.5 Overall Risk/Benefit Assessment *33*
2.5 PHASE I EXPERIENCE IN SOLID TUMORS 33
2.6 JUSTIFICATION FOR ONCE-DAILY DOSING 34
2.6.1.1 Comparison of Schedule Pharmacokinetics: 34
2.6.1.2 Comparison of Schedule Pharmacodynamics: 34
2.6.1.3 Comparison of Schedule Toxicity: 35
3 AFATINIB DRUG PROFILE 36
Table 5: In vitro activity of afatinib (BIBW 2992) in human tumor cell models expressing wild-type or mutated EGFR 37
3.1 PHARMACOLOGY AND TOXICOLOGY PROFILE 37
3.2 CLINICAL EXPERIENCE: AFATINIB 39
3.2.1 Overview *39*
Table 6: Early phase development of BIBW 2992. Adapted from [142] 40
3.2.2 Dose Selection for Single-Agent Trials of Afatinib *40*
3.2.3 Adverse Events with Afatinib *41*
3.2.4 Clinical Efficacy *41*
3.2.5 Pharmacokinetics *43*
3.2.6 Phase II/ III Trials *44*
Table 7: Afatinib Trials in patients with NSCLC: Adapted from [142], [70] *44*
3.3 AFATINIB – COMBINATION-SPECIFIC SAFETY MEASURES 44
3.4 CONCLUSIONS FROM PHARMACOKINETICS IN HUMANS 45
3.5 AFATINIB FORMULATION 46
3.6 DOSAGE, ADMINISTRATION, AND STORAGE 47
4 STUDY DESIGN 47
4.1 OVERVIEW 47
5 SELECTION OF PATIENTS 48
5.1 INCLUSION CRITERIA 48
5.2 EXCLUSION CRITERIA 49
6 TREATMENTS 51
6.1 STUDY CALENDAR – SEE STUDY CALENDAR SECTION 51
6.2 TREATMENTS ADMINISTERED 52
6.2.1 Screening Evaluation *52*
6.2.2 Cycle 1 ..... *53*
6.2.3 Cycle 2 ..... *53*
6.2.4 Therapy Beyond Cycle 2 ..... *53*
6.2.5 End of Treatment visit (EOT) *54*
6.3 DOSE ESCALATION RULES 54
6.3.1 Dose Escalation Schema *54*
Table 8: Dose Escalation Schema 54
6.3.2 Definition of Dose-Limiting Toxicity (DLT) *56*
6.4 CONCOMITANT THERAPY 56
6.4.1 Prohibited and Restricted Therapies During Study *56*
6.4.1.1 Prohibited Therapies 56
6.4.1.2 Restricted Therapies 58
6.4.2 Potential for Afatinib / Dasatinib Interactions *59*
Dasatinib and P-glycoprotein 60
6.5 TREATMENT OF PERSONS OF CHILDBEARING POTENTIAL 60

6.6 PATIENT WITHDRAWAL CRITERIA ... 61
6.7 NONCOMPLIANCE ... 62
6.8 ACCOUNTABILITY PROCEDURES ... 62
6.9 STUDY PERIOD ... 62
6.10 TRIAL DISCONTINUATION ... 63
6.10.1 Special Situations ... *63*
6.11 MANAGEMENT OF ADVERSE EVENTS ... 63
6.11.1 Rash ... *63*
6.11.2 Diarrhea ... *65*
6.11.3 Nausea and Vomiting ... *66*
6.11.4 Pulmonary ... *66*
6.11.5 Fluid Imbalance ... *67*
6.11.6 General Dose Modifications Beyond Cycle 2 ... *67*

7 OBSERVATIONS ... 68

7.1 METHODOLOGY ... 68
7.1.1 Plasma Collection and Processing ... *68*
7.2 CORRELATIVE SCIENCE ... 68
7.2.1 Stored Plasma Samples ... *68*
7.2.2 Radiomic Analysis for Imaging Predictive Markers ... *68*
7.2.2.1 Assessment ... 69
7.2.2.2 Data Recording ... 69
7.3 ASSESSMENT OF SAFETY ... 70
7.3.1 Safety Evaluation Overview ... *70*
7.3.2 Adverse Events ... *71*
7.3.3 Time Period Reporting Requirements ... *72*
7.3.3.1 Hospitalization ... 72
7.3.3.2 Worsening of pre-existing conditions ... 72
7.3.3.3 Assessment of healthcare resource use ... 73
7.3.3.4 Laboratory investigations ... 73
7.3.3.5 Physical examination and performance score ... 74
7.3.3.6 Vital signs ... 74
7.3.3.7 EKG ... 74
7.3.3.8 Left ventricular function assessment ... 74
7.3.4 Data and Safety Monitoring Committee (DMSC) ... *75*
7.4 ASSESSMENT OF EFFICACY ... 75
7.4.1 Evaluation of Response ... *75*
7.4.2 Response Criteria for Phase IA ... *76*
7.4.2.1 Baseline criteria ... 76
7.4.2.2 Specifications by methods of measurements ... 76
7.4.2.3 Tumor response evaluation ... 77
7.4.3 Response Criteria for Phase IB ... *78*
7.4.3.1 RECIST v.1.1 criteria ... 78
Table 10: Best response ... 79
7.4.3.1.1 **Definition of Evaluable Participants**: ... 80
Participants are considered evaluable who receive their first dose of therapy. Non-evaluable participants are those who do not receive the first dose of therapy. Participants who withdraw consent or discontinued due to protocol violations prior to the first evaluation are not considered evaluable for efficacy. ... 80
For other definitions, see Section 7.4.3.2.1 “Progression Definitions”. ... 80
7.4.3.2 Confirmatory measurement / duration of response ... 80
7.4.3.2.1 Progression Definitions ... 81
7.4.4 Reporting of Results ... *82*

8 STUDY DRUG(S) SPECIFICATIONS ... 82

8.1 PRODUCT IDENTIFICATION ... 82
8.1.1 Dasatinib ... *82*

8.1.2 Afatinib ... *82*
8.2 Packaging and Labeling ... 83
8.3 Handling and Dispensing of Investigational Product ... 83
8.4 Investigational Product Records at Investigational Site(s) ... 84
8.5 Return and Destruction of Investigational Product ... 85
8.5.1 Return of Investigational Product ... *85*
8.5.2 Destruction of Investigational Product ... *85*

9 ADVERSE EVENTS & DATA SAFETY MONITORING COMMITTEE (DSMC) ... 86

9.1 Specification of Safety Variables ... 86
9.1.1 Definition of Adverse Events ... *86*
9.1.2 Protocol-Specified Significant Events ... *87*
9.1.3 Serious Adverse Events ... *87*
9.2 Methods and Timing for Assessing AND Recording Safety variables ... 88
9.2.1 Adverse Event Reporting Period ... *89*
9.2.2 Assessment of Adverse Events ... *89*
Table 11: Adverse Event Grading (Severity) Scale ... 89
9.2.3 Data Safety and Monitoring Board ... *91*
9.3 Adverse Event Reporting Guidelines ... 91
9.3.1 MedWatch 3500A Reporting for Good Clinical Practice ... *91*
9.3.2 Responsibilities for SAE reporting to Collaborators ... *91*
9.4 Procedures for Eliciting, Recording, and Reporting Adverse Events ... 92
9.4.1 Expedited Reporting Requirements for Serious Adverse Events ... *93*
9.4.2 Type and Duration of Follow-up of Patients after Adverse Events ... *93*

10 STATISTICAL METHODOLOGY ... 93

10.1.1 Progression Definition: ... *93*
10.1.2 Power Analysis: ... *94*
10.1.3 Subgroup Analysis ... *94*
10.1.4 Safety analysis ... *95*

11 ADMINISTRATIVE SECTION ... 95

11.1 Risk to Subjects ... 96
11.2 Adequacy of Protection Against Risk ... 96
11.3 Importance of the Knowledge to be Gained ... 96
11.4 Data Safety and Monitoring Plan ... 96
11.5 Initial and Ongoing Monitoring and Review ... 97
11.6 Good Clinical Practice ... 99
11.7 Suspension and Termination ... 100
11.8 Institutional and Individual Conflicts of Interest ... 100
11.9 Records Retention ... 101
11.10 Contributions ... 101

12 APPENDICES ... 102

12.1 Appendix 1: Toxicity tables ... 102
Adverse Event Management Guideline: **Papulopustular Skin rash** ... 102
Adverse Event Management Guideline: **Diarrhea** ... 103
Adverse Event Management Guideline: **Localized Edema** ... 104
Adverse Event Management Guideline: **Nausea and Vomiting** ... 104
12.2 Appendix 2: Supplemental Pharmacovigilance Agreement ... 106
12.3 Appendix 3: Supplemental Manufacturing Agreement ... 110

13 LIST OF ABBREVIATIONS ... 116

14 REFERENCES ... 117

PROTOCOL SYNOPSIS

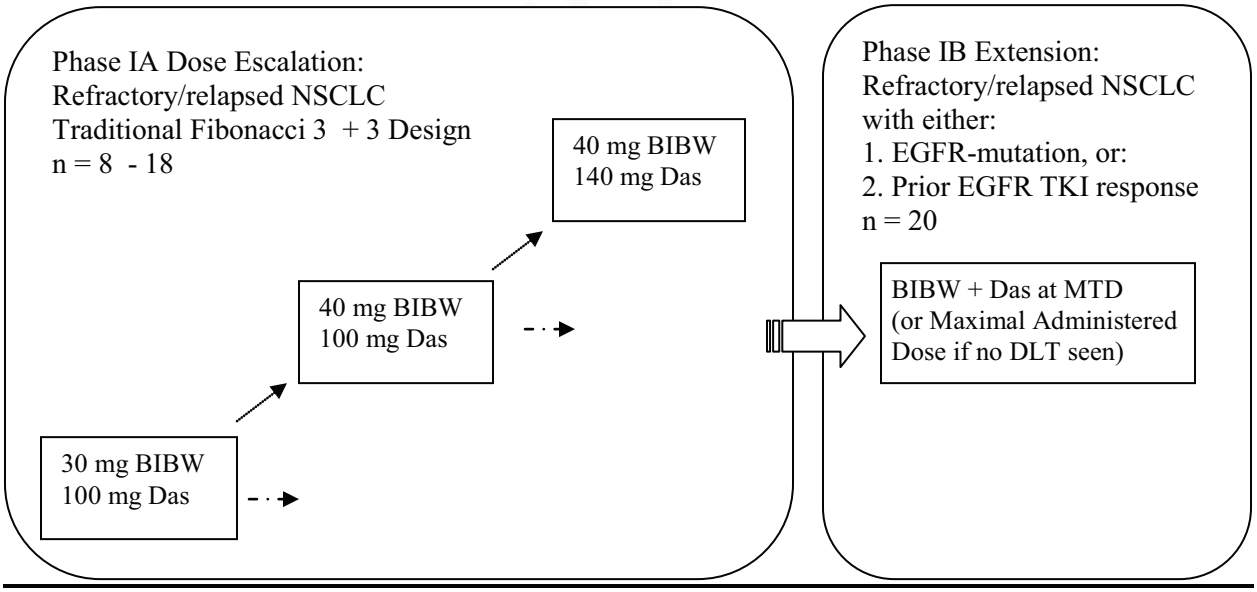
Rationale:

Afatinib (BIBW 2992) is an oral irreversible inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase. This novel agent may prolong survival in patients with advanced non-small cell lung cancer. SRC tyrosine kinase proteins can cooperate with EGFR and can affect tumor cell growth, survival, invasion, and angiogenesis. For these reasons, combined EGFR and SRC tyrosine kinase inhibition may be superior to either agent alone. In EGFR-resistant animal models, afatinib in combination with dasatinib produced synergistic reduction in tumor volume at safe and achievable concentrations in humans. Therefore, a combination Phase I trial is rational in human subjects.

Design:

Phase I trial of the irreversible EGFR inhibitor, afatinib, with the SRC tyrosine kinase inhibitor dasatinib in patients with advanced stage (Stage IIIB/IV disease) Non-Small Cell Lung Cancer (NSCLC).

Figure 1: Flow chart of design



Treatment Regimen:

Afatinib once daily oral tablet, starting Day # 1

Dasatinib once daily oral tablet starting Day #8

Toxicity Evaluation every 7 days for weeks 1-4

Response Evaluation Day #50 ±7 days, then every 56 days ±7 days

Objectives:

Primary Objectives:

1) Determine the safety and tolerability of afatinib in combination with dasatinib in patients with advanced NSCLC.

2) Determine the maximum tolerated dose (MTD) of afatinib in combination with dasatinib.

Secondary Objectives:

1) Estimate the objective response rate (complete response [CR] and partial response [PR]), and clinical benefit rate, in participants with acquired EGFR resistance.

2) Estimate the 6-month progression free survival rate in participants with acquired EGFR resistance.

3) Explore the utility of imaging features as a biomarker for clinical activity of tyrosine kinase inhibitors.

Sample Size:

Total sample size: 28 – 38, as follows:

1. Phase IA Dose-Escalation: n = 8 -18, depending on DLT.
2. Phase IB Extension: n = 20.

Expected accrual is 2 - 3 patients per month; estimated time to complete is 18 months. Accrual rate will be monitored and if accrual rate falls below 75% of expected rate a corrective action plan will be developed.

STUDY CALENDAR – PHASE IA

Cycle length: 28 days		Cycle 1			Cycle 2		Cycle 3 & subsequent [6]	End of Treatment Visit (EOT) [7]
	Screen [8]	D1	D8 ±3	D15 ±3	D1 ±3	D22	D1	
Informed Consent	X							
Toxicity Assessment		X	X	X	X		X	X
Pill Diary Check				X	X		X	
Tumor Evaluation [1]	X [2]					X [13]		
CBC, CMP, Mag.[12]	X [3]	X [9]	X	X	X		X	
EKG	X [2]			X				
LVEF [4]	X [2]						X [5]	
Complete Physical Exam	X [3]		X	X	X		X	X
Vital Signs and PS evaluation [11]	X		X	X	X		X	X
Beta-HCG [10]	X							
Plasma sample		X			X			
Afatinib								
Dasatinib								

Calendar Foot Notes:

1. CT Scan of Chest, Abdomen (SOC) (every 56 days +/- 7 days after C2D22) per Section: Evaluation of Response. .
2. Performed within 28 days prior to enrollment.
3. Performed within 14 days prior to enrollment. See Section 6.1 for additional information.
4. MUGA or ECHO. See Section: Treatments Administered for additional information.
5. Performed every 3 months +/- 14 days after cycle 3 for 1 year or until End of Treatment (EOT) visit.
6. Every 28 days +/- 7 days until toxicity or progression, for 1 year, and every 3 months thereafter.
7. Performed within 21 days of cessation of study drug
8. Screening performed up to 28 days before C1D1.
9. C1D1 labs are excluded if performed within 14 days of screening.
10. Premenopausal females only.
11. Vital Signs include Height, Weight, and include BP, pulse, and respiratory rate after 2 minutes supine rest.
12. See Section: Laboratory Investigations for additional information.
13. C2D22 +/- 7 days

STUDY CALENDAR – PHASE IB

Cycle length: 28 days		Cycle 1		Cycle 2		Cycle 3 & subsequent [7]	End of Treatment (EOT) visit [8]
	Screen [9]	D1	D15 ±3	D1 ±3	D22	D1	
Informed Consent	X						
Toxicity Assessment		X	X	X		X	X
Pill Diary Check				X		X	
Tumor Evaluation [1]	X [2]				X [13]		
CBC, CMP, Mag. [12]	X [3]	X [10]	X	X		X	
EKG	X [2]		X				
LVEF [4]	X [2]					X[6]	
Complete Physical Exam	X [3]			X		X	X
Vital Signs and PS assessment [11]	X			X		X	X
Beta-HCG [5]	X						
Plasma sample		X		X			
Afatinib							
Dasatinib							

Calendar Foot Notes:

1. CT Scan of Chest, Abdomen (SOC) (every 56 days +/- 7 days after C2D22) per Section: Evaluation of Response.
2. Performed within 28 days prior to enrollment
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11. Vital Signs include Height, Weight, and include BP, pulse, and respiratory rate after 2 minutes supine rest.
12. See Section: Laboratory Investigations for additional information.
13. C2D22 +/- 7 days

1 INTRODUCTION

1.1 Significance

1.1.1 Scope of the Problem: Treatment of NSCLC

Approximately 222,500 patients in the United States are diagnosed with lung cancer yearly, of which 85% is classified as non-small cell lung cancer (NSCLC) [8, 9]. Eighty-three percent will eventually die of their disease [9]. In addition, NSCLC remains the leading cause of death related to cancer worldwide [10]. Until 2009, platinum-based doublets have been standard therapy for the majority of advanced NSCLC based upon moderate improvement in survival and quality of life [11-14]. However, overall survival (OS) has reached a plateau even with the enhancement of these regimens and development of the second line chemotherapy in NSCLC [12, 15, 16]. Therefore, NSCLC is a pervasive clinical problem for which additional treatment strategies are urgently needed.

1.1.2 Epidermal Growth Factor Receptor (EGFR) Inhibitors in Lung Cancer

EGFR is a member of the ErbB family of receptor tyrosine kinases. It is frequently overexpressed and negatively correlated with prognosis in many types of human malignancy, including NSCLC [17, 18]. EGFR is a 170-kDa plasma membrane glycoprotein composed of an extracellular ligand-binding domain, a transmembrane region, and an intracellular tyrosine kinase domain with a regulatory COOH-terminal segment [19]. Binding of ligand to EGFR induces not only homo-dimerization with EGFR but also hetero-dimerization with the other members of the ErbB family of receptor tyrosine kinases such as Her2 (ErbB2), Her3 (ErbB3), and Her4 (ErbB4) [20]. The receptor dimerization results in consequent conformational changes, following activation of the receptor kinase and auto-phosphorylation of specific tyrosine residues within the COOH-terminal region of the protein [19, 21, 22]. These events trigger intracellular signaling pathways such as those mediated by the protein kinases Akt or extracellular–signal regulated kinase (Erk), both of which play fundamental roles in the control of numerous cellular processes [23-26]. In response to ligand binding, the ligand-EGFR complex is rapidly internalized, allowing EGFR to interact with various signaling proteins such as Grb2 or PI3K, and reach full and sustained activation of Akt or Erk signaling [27-29]. Thereafter, EGFR is either recycled back to the cell surface or proteolytically degraded (Fig. 1). Recognition of the role of EGFR in oncogenesis has led to the development of EGFR-targeted therapies including small-molecule TKIs such as gefitinib and erlotinib. Both of these

molecules compete with adenosine triphosphate (ATP) at the intracellular tyrosine kinase domain [30, 31].

1.1.3 EGFR: A Driver Mutation in NSCLC

The epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) have been applied to the treatment of NSCLC [32-35] following the discovery of the biological significance of EGFR in this cancer [17, 18]. In 2004, 3 groups reported that NSCLC patients with EGFR mutation experience a dramatic response to gefitinib or erlotinib [36-38]. Both deletions in exon 19 and a point mutation that substitutes an arginine for a leucine at codon 858 (L858R) in exon 21 are known to be the most common EGFR mutations [39-41]. Study participants with exon 19 deletion had longer survival than those with L858R point mutation [46, 47]. The presence of EGFR mutation activates the receptor tyrosine kinase by disrupting autoinhibitory interactions [30] and induces higher phosphorylation of EGFR compared with wild-type EGFR [36, 42]. In addition, NSCLC cells with EGFR mutation constitutively activate both EGFR and downstream signaling because of ligand-independent dimerization of the receptor [43-45]. EGFR mutation allows gefitinib to bind more tightly to EGFR compared with the wild-type EGFR [30, 42]. These results indicate that cells with specific EGFR mutations depend upon EGFR signaling for growth and differentiation and have particular sensitivity to EGFR-TKIs such as gefitinib.

Several recent phase III trials, WJTOG3405 [46], NEJ002 [47], or subset analysis of IPASS [48, 49] demonstrated that single agent gefitinib provided superior response rates and progression-free survival (PFS) to platinum-based doublets by selecting for participants harboring EGFR mutation. Subset analysis of IPASS trial further revealed that EGFR mutation, and not copy number, is the best predictor of gefitinib response [48, 49]. Despite promising initial response rates, the duration of response to EGFR-TKI is almost inevitably limited by the emergence of acquired drug resistance.

1.1.4 T790M Gate-keeper *EGFR* Mutation and Irreversible EGFR-TKI in NSCLC

Although NSCLC patients with EGFR mutation show an initial dramatic response to EGFR-TKIs such as gefitinib or erlotinib, almost all acquire resistance to these drugs within one to two years. Specifically, a secondary point mutation in exon 20 of EGFR that substitutes methionine for threonine at amino acid position 790 (T790M) was identified in the NSCLC patients who developed acquired resistance to gefitinib or erlotinib [50, 51]. About 50% of NSCLC patients with acquired resistance to EGFR-TKIs have T790M secondary mutation [52, 53]. T790M is located in the ATP-binding cleft of the EGFR structure and is thought to block EGFR-TKI binding due to alteration of the topology causing steric hindrance [31, 50, 51, 54, 55]. Furthermore, the presence of T790M confers a growth advantage to the cells both in vitro

and in vivo [56], suggesting that T790M itself has oncogenic effects by enhanced kinase activity [42]. Affinity of ATP-binding cleft may be increased by the presence of T790M [57]. This may partially explain how irreversible EGFR-TKIs such as CL387,785 [58, 59], PF00299804 [60, 61], afatinib (BIBW 2992) [62], or HKI-272 [63] overcome the resistance induced by T790M (Fig. 2a). However, irreversible EGFR-TKIs have only limited ability to overcome acquired resistance, [59, 60, 64] and may actually induce their own resistance in T790M mutated cancer cell lines [65-69]. In line with these preclinical results, afatinib (BIBW 2992) did not show prolonged OS in patients with advanced NSCLC, whose disease progressed after receiving chemotherapy and an EGFR-TKI (LUX-Lung 1 Phase IIb/III trial) [70]. These results suggested that single-agent irreversible EGFR-TKI is not enough to reverse acquired resistance completely. However, other agents combined with irreversible EGFR-TKIs may hold promise in overcoming T790M and other acquired resistance mutations [64, 71, 72]. The identification of alternative therapy to single agent EGFR-TKI or strategies capable of overcoming acquired resistance to EGFR-TKI is thus urgently needed.

1.1.5 Mechanisms of EGFR-TKI Resistance in NSCLC

MET gene amplification has recently been identified as a novel mechanism of gefitinib resistance and is detected in 22% of tumor samples from NSCLC patients with EGFR mutations with acquired gefitinib resistance [73]. Both MET and EGFR signaling activate PI3K via ErbB3 (Her3) in gefitinib-resistant HCC827GR cells with MET amplification. This cell line was generated by enforced gefinitib exposure of HCC827 cells harboring an exon 19 deletion. In this situation, the combination of gefitinib and MET inhibitor PHA665752 is required to shut down survival signaling in these cells (Fig. 2b) [73]. Although there are few studies describing alternative therapies to overcome EGFR-TKIs resistance induced by MET amplification, the combination of inhibitors that block the downstream molecules of both EGFR and MET, such as the PI3K inhibitor combined with the MEK inhibitor, could be one strategy (Fig. 2b) [74]. Additional mechanisms of acquired EGFR-TKIs resistance include alteration of insulin-like growth factor 1 receptor (IGF1R) signaling [75-77], the loss of PTEN [78, 79], or hepatocyte growth factor (HGF) overexpression [80]. Recently, epithelial to mesenchymal transition (EMT) has been identified as a primary or acquired resistant mechanism to EGFR-TKI. However, the strategy to overcome EMT-related EGFR-TKI resistance remains unclear [81-85].

1.1.6 Significance of the Src-TKI Dasatinib in NSCLC

The proto-oncogene SRC is involved in the initiation of carcinogenesis and proliferation of NSCLC, since its product mediates signaling between either integrins or receptor tyrosine kinases and their downstream effectors (Fig. 3) [86]. SRC is both an upstream activator and downstream mediator of EGFR, and Src phosphorylation is detected in 33% of NSCLC tumors

[87]. The Src-TKI dasatinib is effective in EGFR mutant NSCLC cells similar to EGFR-TKIs, based on the results showing that Src is activated more in EGFR mutant cells than EGFR wild-type cells [88]. In line with these results, Src-TKI PP1 combined with EGFR-TKI gefitinib shows increased levels of apoptosis in EGFR mutant NSCLC cells [87]. Our chemical and phosphoproteomic characterization identified nearly 40 different kinase targets of dasatinib and showed that SRC, FYN, and EGFR are relevant targets for dasatinib action in NSCLC [89]. A single-arm phase II trial of participants with relapsed refractory EGFR mutant or unselected NSCLC demonstrated that the overall disease control rate of single-agent dasatinib was 43%, including partial response (PR) in one patient and prolonged stable disease (SD) in four others [90]. Our recent phase I/II study showed that the combination of dasatinib and EGFR-TKI erlotinib is tolerable with 63% of disease control rate including two PR and one bone response in advanced NSCLC [91]. These results suggest the potential clinical activity of dasatinib in NSCLC treatment.

Table 1: Drugs that may overcome EGFR resistance. Adapted from [92]

Drug	Manufacturer	Mechanism of action	Recommended single-agent dose	Phase
Afatinib	Boehringer Ingelheim	Irreversible TKI	40 mg daily	3
PF299	Pfizer	Irreversible TKI	45 mg daily	3
HKI292	Pfizer	Irreversible TKI	240 mg daily	2
ARQ197	ArQule/Daiichi Sankyo	Non-competitive c-Met inhibitor	360 mg bid.	3
Met/Mab	Roche/Genentech	Met antibody	15 mg/kg q.3.w	3

bid.=twice daily administration; q.3.w.=every three weeks;

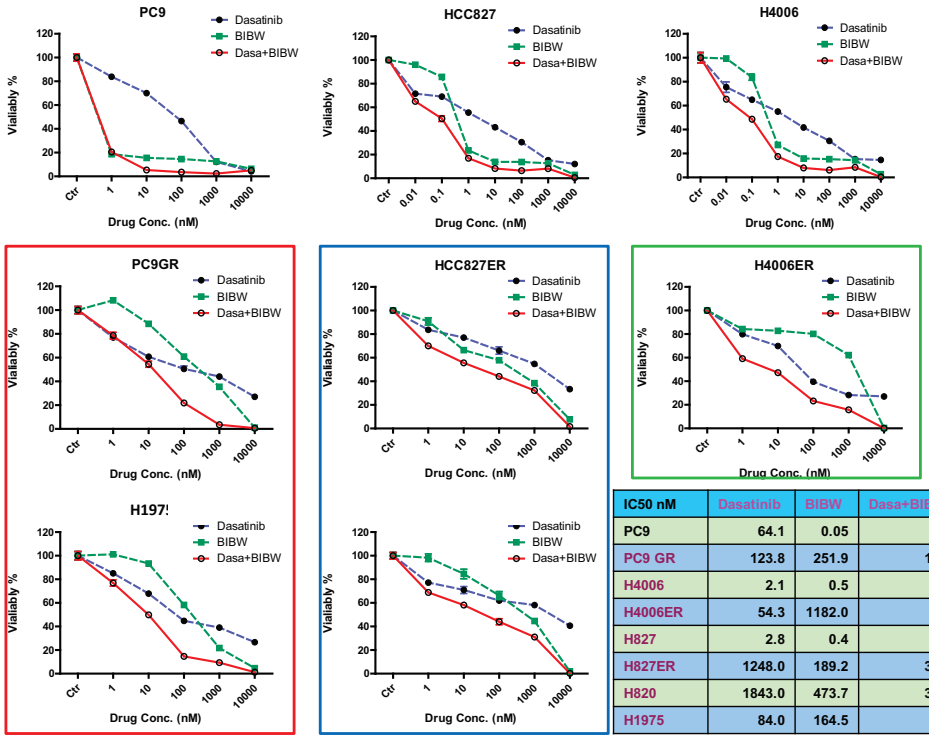
1.1.7 SRC Signaling in Non-Small Cell Lung Cancer

Members of the Src family of protein tyrosine kinases (SRC) can link signaling initiated by growth factor, integrin, and cytokine receptors on the surface of cells to their downstream effector signaling cascades (Fig. 1, next page) [93]. Upon activation by receptor tyrosine kinases through SH2 binding, SRC is auto-phosphorylated at tyrosine 419. SRC cooperates with multiple receptor tyrosine kinases to modulate signaling [94]. As one example relevant for lung cancer, c-Src cooperates with the epidermal growth factor receptor (EGFR) and Src-kinase activity is required for transformation by EGFR.

SRC signaling can result in activation of downstream signaling pathways that control cellular proliferation, survival, invasion and metastasis, and angiogenesis (Figure 1). SRC signaling can allow for enhanced cellular proliferation by upregulating genes important in cell cycle progression such as Myc, $p21^{WAF1/CIP1}$, cyclin D, and $p27^{Kip1}$, and cdc2 [95, 96]. SRC protect cells against apoptosis induced by loss of cell adhesion and PI3K/Akt and Stat3 are key downstream survival cascades regulated by SRC. SRC promotes tumor cell invasion and metastasis by affecting cell adhesion, invasion, and motility [97]. C-Src can regulate both focal adhesions and adherens junctions necessary for invasion of tumor cells by activation of downstream focal adhesion kinase (FAK), $p130^{Cas}$, and paxillin [97]. Vascular endothelial growth factor (VEGF) and hypoxia inducible factor 1-alpha (HIF1-a) are downstream targets for c-Src, and small molecule inhibitors of SRC can negatively regulate VEGF and inhibit angiogenesis [98-102].

Elevated SRC activity is found in human tumors, including lung cancer, resulting from diverse mechanisms including tyrosine phosphatase-mediated dephosphorylation of Tyr 527, increased SRC protein levels, increased upstream receptor tyrosine kinase activity, or loss of negative regulatory proteins [97, 103, 104]. Small molecule inhibitors of SRC have been shown to have antitumor properties through negatively regulating cell proliferation, survival, angiogenesis, and invasion [105-107]. A number of small molecule oral SRC tyrosine kinase inhibitors have entered early-phase clinical trials. One such SRC tyrosine kinase inhibitor, dasatinib (BMS-354825), is a novel bioavailable tyrosine kinase inhibitor that has recently completed phase I clinical trials in patients with solid tumors. Dasatinib has been shown to exert antitumor effects in lung cancer cells, and preclinical data suggest that dasatinib can inhibit tumor growth and induce tumor cell death in a subset of non-small cell lung cancer cell lines dependent on EGFR for growth and survival [88, 107].

Based on the importance of EGFR signaling in lung cancer, the known cooperation between EGFR and SRC proteins, and evidence of elevated SRC activity in human lung cancers, we propose a phase I trial of combined afatinib and dasatinib in patients with advanced non-small cell lung cancer.



IC50 nM	Dasatinib	BIBW	Dasa+BIBW
PC9	64.1	0.05	0.4
PC9 GR	123.8	251.9	12.1
H4006	2.1	0.5	0.1
H4006ER	54.3	1182.0	5.5
H827	2.8	0.4	0.1
H827ER	1248.0	189.2	31.3
H820	1843.0	473.7	38.3
H1975	84.0	164.5	8.8

Figure 2. EGFR-mutant NSCLC in vitro results from Haura lab

1.2 Combination of Afatinib with Dasatinib

Given the limited activity of irreversible EGFR-TKI [59, 60, 64-70], the identification of new targets to overcome EGFR-TKI resistance in NSCLC is urgently needed. We have previously shown that the Src inhibitor dasatinib effectively inhibits cell growth in the gefitinib-resistant HCC827GR cells with MET amplification based on the results showing that Src acts downstream of both EGFR and MET in these cells [108]. Another report demonstrated that combination therapy including dasatinib has activity in Lkb-1 deficient NSCLC mice model with EMT phenotype, suggesting that Src is a possible target against EGFR-TKI resistance caused by EMT [109]. To investigate how signal transduction is altered in NSCLC cells with T790M, we generated PC9GR cells with T790M by long-term gefitinib exposure of PC9 cells with exon 19 deletion. Global phospho-proteomics approach revealed that the activation of Src family kinases were persistent even with erlotinib treatment in PC9GR cells, suggesting that Src family kinases compensate EGFR inhibition in NSCLC cells with T790M. Western blot assays demonstrated that the irreversible EGFR-TKI afatinib did not alter the activation of Src family kinases in PC9GR and H1975 NSCLC cells with T790M. The Src inhibitor dasatinib combined

with afatinib abolished this Src phosphorylation along with complete suppression of phospho-Akt and Erk.

In addition, significant PARP cleavage was observed in these cells, suggesting apoptotic cell death. Consistent with the effects on cell signaling and apoptosis, dasatinib enhances anti-tumor activity of afatinib in both GLO cell proliferation assay and caspase-3 apoptosis assay in PC9GR and H1975 cells. The combination of afatinib and dasatinib showed significant in vivo tumor regression in PC9GR xenograft studies. Furthermore, we also demonstrated that this combination improved IC50 dose even in HCC827ER and H820 cells with MET amplification as well as in H4006ER cells with EMT phenotype. Although single-agent dasatinib has no activity in patients with EGFR-mutant NSCLC with acquired resistance to EGFR-TKI [110], our preliminary results suggest a role for Src in maintaining downstream signaling despite irreversible EGFR inhibitors and support further studies of irreversible EGFR combined with dasatinib in NSCLC patients who acquired resistance to EGFR-TKI.

1.3 Cancer Radiomics

Adapted from Gillies and Gatenby U01 CA 143062 Progress Report 2011.

The enormous progress in understanding the molecular causes and pathophysiology of cancer has not generally translated into improved outcomes. One widely advocated strategy for improving cancer therapy is development of personalized cancer medicine in which treatment is tailored for individual patients. As cancers are dynamical systems, this will require measurement tools with the ability to repeatedly measure key parameters accurately over time.

1.3.1 Scope of Imaging Problem

Clinical imaging has the key characteristics necessary for development of personalized cancer therapy, but many questions remain: How much useful data can be obtained from clinical images? Can image feature data be used to better prognose outcome regardless of therapy or predict response to specific therapies? Are there key imaging parameters that can serve as early biomarkers for tumor response or non-response to chemo- or radio-therapies? If radiology is to play an integral role in personalized cancer therapy, it will require acquisition of images with the highest possible quality (lowest inter-test variance), and extensive analysis that will bridge imaging features to quantitative molecular properties of tumors and eventually, outcome. Historically, clinical imaging approaches have tended to be descriptive and qualitative, characterizing tumors as, e.g. “enhancing, irregular borders, central necrosis” etc. These features have to be made more quantitative to match them to more quantitative measures of tumor biology, such as gene expression patterns. Currently, the only two

commonly used quantitative measures in tumor radiology are tumor size (RECIST or bidimensional measures) and standard uptake value (SUV) in FDG-PET scanning. Thus, Gillies Lab propose a detailed examination of additional quantitative features that are extractable from CT scan images from the patients in this trial, with the hypothesis that these additional features will allow quantitative predicting or monitoring of response. Critical to the success of this approach is that it will generally use standard of care scans, thus will not require scheduling of additional patient visits or incur additional costs beyond analysis. The ultimate goal will be analysis of these features to determine which combinations can be used to predict clinical outcomes in conjunction with, or in lieu of, the additional molecular biomarkers planned for this study.

Table 1.3: Potentially Representative CT features and Variables.

Feature	Calculation	Values for patient*	Hypothesis
1a. Area and 1b. Perimeter	a. Area/Number of pixels containing tumor b. Perimeter of the ROI with snake.	Area = 1201 mm^2 (875 voxels) Perimeter = 152.4 mm	Larger tumors are later stage and area is a good measure of size.
2a. Volume 2b. Surface area	a. 3D reconstruction Sum of number of pixels in tumor in all slices. Region growing b. Surface area with 3D snake.	*Volume = 2615 mm^3 *Surface area = 1099.3 mm^2	3-D volume is a better measure of size than is 2-D area.
3. Surface to volume ratio	2b divided by 2a	*S/V ratio = 0.42mm^{-1}	Lesions with higher S/V are more lobulated and perhaps more invasive, less responsive/worse outcome
4. Margin gradients	Attenuation gradient across 5 pixels at the edge of the tumor. Measure every 5 degrees from centroid. Express as (a) average value (HU per mm) ± SD, and (b) spatial plot for regional variations	Slope (mean± SD) = 133.5± 31.3 HU/mm See Figures A and B	Lesions with diffuse margins (low gradient) have increased partial volumes at edge and should also be a characteristic of having small spiculations and more invasive.
5. Macro spiculations	Number of observed spiculations	Number of macro spiculations = 8	Spiculated tumors are more aggressive and less responsive
6. Intensity at edge and core of tumor	Average ± SD of HU in core and rim. Core defined as sphere with radius equal to 0.25 of the total lesion radius. Rim is coaxial between radii of 0.7 and 0.9. Express scalar and ratio values.	See below	Volumes with low attenuation may represent necrotic areas and thus may be less responsive.
7. Hetero-geneity	a. Mean, SD, range of intensities over entire volume of lesion b. Histogram Analysis: descriptors like skewness and kurtosis can be derived from the 2-component fit, e.g. gamma (mu, nu)	*Mean (mean ± SD) = 19.33 ± 76.59 HU *Minimum = -249 HU *Maximum = 118 HU *Variance = 5866.30 Skewness = -1.06 Kurtosis = 7.15 (See Figure C)	More heterogeneous lesions may contain 'hot spots' of resistant volumes.
8. Attachment to pleural wall	a. Attachment b. Degree of attachment/ ratio of free to attached surface area	Not attached to pleural wall	Lesions attached to pleural wall have greater propensity to metastasize.
9. Shape	a. Fractional anisotropy (f.a.) of long vs. short axes. b. Circularity c. Avg ± SD and range of distance form center of mass to edge	Circularity = 0.83 Distance from center to edge (mean ± SD) = 19.97 ± 3.24 mm	Oblong tumors are growing along existing vasculature and are thus more likely to have mediastinal or distant metastases.
10. Air spaces within the tumor	a. Total fractional volume of the air spaces b. Number of spots c. Size distribution (mean and SD) of spots	Relative volume of air inclusions = 0.21 Number of air inclusions = 110 Volume of air inclusions (mean ± SD) = 4.89 ± 8.35 mm	Black spots represent necrosis which would be a negative predictor for outcome.
*calculated using Definiens, otherwise manually measured.			

A critical component of this approach is the application of methods to efficiently extract quantifiable information from radiologic images, i.e. “radiomics.” Insofar as possible these methods should be semi-automated wherein the radiologist identifies the lesion and computer software proceeds to segment, render and generate a report of quantitative features. From these reports, Gillies Lab can ask the following questions: Which features are informative (e.g. have a wide range and be measureable in all samples)? What is the variance from one measurement to another and what are the critical sources of that variance? Are the features with largest dynamic range related to outcomes?

1.3.2 Radiomics: A Rationale Solution

The central hypothesis of cancer “radiomics” is that tumor imaging features reflect underlying gene expression patterns and tumor pathophysiology. This is supported by common sense as well as empirical observation. In the simplest cases, changes in expression of specific genes can affect specific imageable parameters, such as vascular-endothelial growth factor (VEGF) effects on perfusion or survival gene effects on tumor density, both of which are measurable by MRI. Furthermore, the idea that imaging features reflect underlying differences in gene expression is the basis for image-guided biopsy, which has clearly shown that tumors exhibit distinct regional variations in gene expression that are correlated with image features, such as perfusion. Image features also underlie the tremendous power of computer aided diagnosis (CAD) systems, such as those pioneered by Giger and colleagues. The use of image features has been elevated to a new level through the work of Kuo and colleagues, who have compared extractable features from MRI or CT to predict global gene expression patterns in glioblastoma multiforme (GBM) and HCC). In GBM, there are clear correlations between histopathology grade and MR imaging features. However, the diversity of MR phenotypes is greater than that of histology, e.g. tumors of similar histopathology can exhibit distinctly different MR imaging patterns.

In Hepatocellular cancer , Kuo quantified 138 features from contrast-enhanced CT images in a training set from 28 patients. These features were individually filtered according to their frequency in the datasets, interobserver agreement, and independence from other features, resulting in a subset of 32 highly informative features. A modified neural net was then used to identify 116 gene “modules” that contained sets of genes (out of 6732 total) whose variation was coherent. The algorithm then identified combinations of imaging features that were highly correlated with each gene module. This training set was then tested using a permutation of the original data and a completely independent data set. Only 28 of the imaging features were needed to explain all 116 gene modules, and 9 features could explain half of them. For each

gene module, only 3-4 imaging features were needed. Thus, CT feature data can be used to predict global gene expression.

Since beginning of 2010, with support from the NCI Quantitative Imaging Network (QIN), Gillies group has been performing Radiomic analyses of NSCLC. In these studies, Gillies Lab have employed a commercial platform supplied by Definiens™ to segment, render and report. Gillies Lab have modified the software so that the segmentation is operator-independent, and these have been compared to lesions that have been manually segmented by radiologists and radiation oncologists in two different studies. With segmented images, Gillies Lab have generated a feature set to contain up to 233 quantified 2-D and 3-D features, including shape, texture, attenuation and size features. A test-retest study has shown that a large number (>160) of these features have high reproducibility with a broad natural range. A subset of these have been used to develop classifiers in NSCLC to identify broncho-alviolar carcinoma from squamous and adenocarcinomas. Studies are currently ongoing to determine if these features can be quantitatively related to gene expression or outcomes. To this end, Gillies Lab have extracted image features from 460 patient scans along with gene expression arrays. These are currently being combined into a database for informatic and statistic analyses.

NSCLC in the US has one of the highest mortality rates and patients are routinely imaged with CT scans for staging and monitoring, respectively. Thus, any advance in the ability to predict response and individualize treatment will have great impact. In the present study, we propose to extend this radiomic analysis application in a prospective way to NSCLC patients being treated with tyrosine kinase inhibitors. These features will be compared to PFS outcome in the experimental arm of the study independently and combined.

Our goal is to provide accurate results with minimal subjective medical interaction. A crucial component is to have the appropriate features to accurately and quantitatively measure lesions.

Thus, the segmentation and report generation will follow much the same protocol as the current NSCLC trial. However there are two major deviations in the current study that are important to the development of the Radiomic approach. First, the current study will be performed in a multicenter setting. Thus the image capture must be standardized. Second, the current study will invite comparison between pre- and post-therapy CT scans to identify quantitative and objective predictors of response that may be more sensitive than the standard RECIST 1.1 measures. Such “change metrics” add an additional yet tractable element of complexity that may be rich in information.

2 DASATINIB DRUG PROFILE

Dasatinib (SPRYCEL®) is a potent, broad-spectrum ATP-competitive inhibitor of 5 critical oncogenic tyrosine kinase/kinase families: BCR-ABL, SRC, c-KIT, PDGF receptor β (PDGFRβ), and ephrin (EPH) receptor kinases, each of which has been linked to multiple forms of human malignancies[1].

Drug discovery and nonclinical pharmacology studies showed that dasatinib[2]:

- Kills BCR-ABL dependent leukemic cell lines, including a number that are resistant to imatinib due to kinase domain mutations or overexpression of SRC family kinases and is effective against all imatinib-resistant kinase domain mutations tested to date, except T315I.
- Inhibited proliferation of cancer cell lines that express activated SRC or c-KIT.
- Potently inhibits VEGF-stimulated proliferation and migration in HUVECs.
- Has potent bone anti-resorptive activity.

2.1 Activity in Experimental Models

2.1.1 In Vitro Molecular Studies

Dasatinib potently inhibits SRC kinases, BCR-ABL, c-KIT, PDGFRβ, and EPHA and was less potent against 16 other unrelated protein tyrosine kinases (PTKs) and serine/threonine kinases. Imatinib is less potent against several key enzymes: for example, dasatinib was 260-, 8-, 60-, and >1000-fold more potent than imatinib versus BCR-ABL, c-KIT, PDGFRβ, and SRC kinases, respectively.

In vitro, dasatinib was able to overcome imatinib resistance resulting from BCR-ABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression.

Dasatinib inhibits the BCR-ABL kinase with an in vitro IC_{50} of 3 nM, a potency 260-fold greater than that of imatinib mesylate (IC_{50} = 790 nM). In cellular assays, dasatinib killed or inhibited the proliferation of all BCR-ABL dependent leukemic cell lines tested to date. Dasatinib also demonstrated undiminished anti-tumor activity against several preclinically and clinically derived models of imatinib mesylate resistance. Evidence that SRC family kinase over-expression may play a role in clinical resistance to imatinib mesylate was demonstrated in three CML cell lines established from patients who failed imatinib mesylate therapy. These cells remained highly sensitive to the cell-killing effects of dasatinib.

These results demonstrate that dasatinib is effective in reducing the proliferation or survival of both imatinib mesylate-sensitive and resistant cells, and its inhibitory activity is not solely dependent on BCR-ABL.

2.1.2 Lung Cancer Cell Studies

Two reports have presented preclinical data suggesting antitumor properties of dasatinib in human lung cancer cells [88, 107]. Dasatinib is able to induce cell cycle arrest, induce apoptosis, and inhibit tumor cell invasion in lung cancer cell lines. Cell fate (death versus growth arrest) in lung cancer cells exposed to dasatinib is dependent on EGFR status. In cells with EGFR mutation that are dependent on EGFR for survival, dasatinib reduces cell viability through the induction of apoptosis while having minimal apoptotic effect on cell lines with wild-type EGFR. The induction of apoptosis in these EGFR mutant cell lines corresponds to down-regulation of Akt and Stat3 survival proteins. In cell lines with wild-type EGFR or resistant EGFR mutation that are not sensitive to EGFR inhibition, dasatinib induces a G1 cell cycle arrest with associated changes in cyclin D and p27 proteins as well as inhibits activated FAK and prevents tumor cell invasion. These results demonstrate that dasatinib could be effective therapy for patients with lung cancers through disruption of cell growth, survival, and tumor invasion. Furthermore, these results suggest EGFR status is important in deciding cell fate in response to dasatinib.

2.1.3 In Vivo Studies

The activity of dasatinib against CML cells in vitro was reproduced in vivo against several human CML xenograft models grown subcutaneously in SCID mice. Against the K562/imatinib mesylate/R CML model, dasatinib was curative in 100% of the treated animals. In contrast, at its optimal dose and schedule, imatinib mesylate was inactive. In lung cancer mouse xenograft models, the combination of dasatinb with BIBW2992 was synergistic in inducing tumor volume reduction (Figure 3).

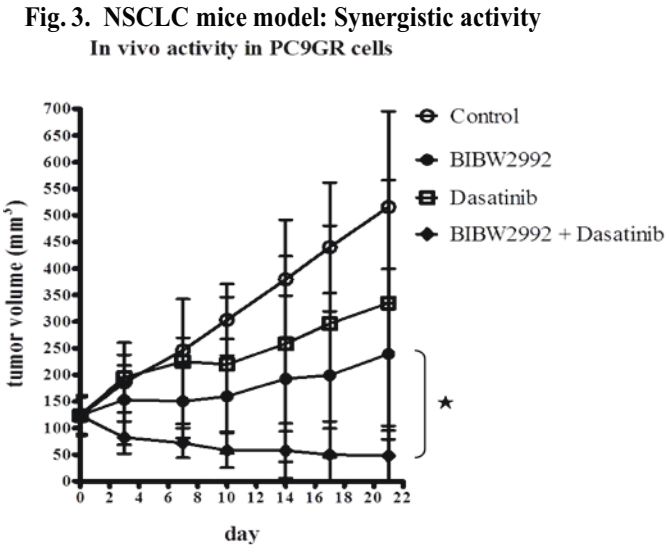


Figure 3. Haura lab mutated NSCLC mouse model: synergistic activity

2.1.4 Preclinical Toxicology

Single or repeated oral administration of dasatinib principally affected the gastrointestinal (GI) tract, including the liver and the hematopoietic and lymphoid systems in rats and monkeys. Other prominent effects after single oral administration of dasatinib included renal and cardiac toxicity in rats at lethal doses and cutaneous hemorrhage in monkeys. Dasatinib can also affect the immune system and bone turnover.

Dasatinib in vitro activity in the HERG/IKr and Purkinje-fiber assays indicated a moderate liability for prolongation of cardiac ventricular repolarization (QT interval) in the clinic. However, there were no dasatinib-related changes observed in electrocardiograms, nervous system function, respirations and heart rate, blood pressure, or arterial oxygen saturation in single-dose, 10-day, or 1-month oral toxicity studies in monkeys.

Dasatinib was found to exhibit a profile of broad-spectrum platelet inhibition best typified by anti-platelet agents such as the GPIIb/IIIa antagonists, integrelin and abciximab.

Finally, modulation of SRC kinase activity could also affect osteoclast morphology and function and bone remodeling. This effect could potentially result in an increase in bone mineral density and a phenotype analogous to osteopetrosis.

2.2 Clinical Pharmacokinetics

The pharmacokinetics of dasatinib has been evaluated in 229 healthy subjects and in 137 patients with leukemia.

2.2.1 Absorption

Maximum plasma concentrations (C_{max}) of dasatinib are observed between 0.5 and 6 hours (T_{max}) following oral administration. Dasatinib exhibits dose proportional increases in AUC and linear elimination characteristics over the dose range of 15 mg to 240 mg/day. The overall mean terminal half-life of dasatinib is 3–5 hours.

Data from a study of 54 healthy subjects administered a single, 100-mg dose of dasatinib 30 minutes following consumption of a high-fat meal resulted in a 14% increase in the mean AUC of dasatinib. The observed food effects were not clinically relevant.

2.2.2 Distribution

In patients, dasatinib has an apparent volume of distribution of 2505 L, suggesting that the drug is extensively distributed in the extravascular space. Binding of dasatinib and its active metabolite to human plasma proteins in vitro was approximately 96% and 93%, respectively, with no concentration dependence over the range of 100–500 ng/mL.

2.2.3 Metabolism

Dasatinib is extensively metabolized in humans, primarily by the cytochrome P450 enzyme 3A4. CYP3A4 was the primary enzyme responsible for the formation of the active metabolite. Flavin-containing monooxygenase 3 (FMO-3) and uridine diphosphate-glucuronosyltransferase (UGT) enzymes are also involved in the formation of dasatinib metabolites. In human liver microsomes, dasatinib was a weak time-dependent inhibitor of CYP3A4.

The exposure of the active metabolite, which is equipotent to dasatinib, represents approximately 5% of the dasatinib AUC. This indicates that the active metabolite of dasatinib is unlikely to play a major role in the observed pharmacology of the drug. Dasatinib also had several other inactive oxidative metabolites.

2.2.4 Elimination

Elimination is primarily via the feces. Following a single oral dose of [^{14}C]-labeled dasatinib, approximately 4% and 85% of the administered radioactivity was recovered in the urine and feces, respectively, within 10 days. Unchanged dasatinib accounted for 0.1% and 19% of the administered dose in urine and feces, respectively, with the remainder of the dose being metabolites.

2.2.5 Clinical Experience with Dasatinib in CML and Ph+ ALL

Four single-arm multicenter studies were conducted to determine the efficacy and safety of dasatinib in patients with CML or Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) resistant to or intolerant of treatment with imatinib. Resistance to imatinib included failure to achieve a complete hematologic response (within 3–6 months) or major cytogenetic response (by month 12) or progression of disease after a previous cytogenetic or hematologic response. Imatinib intolerance included inability to tolerate 400 mg or more of imatinib per day or discontinuation of imatinib because of toxicity. The studies are ongoing. The results are based on a minimum of 6 months follow-up after the start of dasatinib therapy. Most patients had long disease histories with extensive prior treatment, including imatinib, cytotoxic chemotherapy, interferon, and stem cell transplant. The maximum imatinib dose had been 400–600 mg/day in about one-half of the patients and >600 mg/day in the other half.

All patients were treated with dasatinib 70 mg BID on a continuous basis. The median durations of treatment were between 2.8 - 5.6 months.

The primary efficacy endpoint in chronic phase CML was major cytogenetic response (MCyR), defined as elimination (complete cytogenetic response, CCyR) or substantial diminution (by at least 65%, partial cytogenetic response) of Ph+ hematopoietic cells. The primary endpoint in accelerated phase, myeloid blast phase, and lymphoid blast phase CML, and Ph+ ALL was major hematologic response (MaHR), defined as either a complete hematologic response or no evidence of leukemia as defined in Table 2.

Most cytogenetic responses occurred after 12 weeks of treatment, when the first cytogenetic analyses were performed. Hematologic and cytogenetic responses were stable during the 6-month follow-up of patients with chronic phase, accelerated phase, and myeloid blast phase CML. The median durations of major hematologic response were 3.7 months in lymphoid blast CML and 4.8 months in Ph+ ALL.

There were no age- or gender-related response differences.

2.3 Safety of Dasatinib in Clinical Studies

2.3.1 Safety in Hematologic Malignancy

The data presented in Table 2 reflect exposure to dasatinib in 2182 patients with leukemia in clinical studies (starting dosage 100 mg once daily, 140 mg once daily, 50 mg twice daily, or 70 mg twice daily). The median duration of therapy was 11 months (range 0.03-26 months).

The majority of dasatinib-treated patients (1,864 [85%]) experienced at least 1 drug-related adverse reactions at some time. Drug was discontinued for adverse reactions in 14% (296/2182) of subjects. Drug related AEs leading to discontinuation in any 1 category occurred in ≤ 1% of the subjects with the exception of pleural effusion (85/2182; 4%). In subjects with chronic phase CML, drug-related pleural effusion accounted for discontinuation in 52 of the 1150 subjects. Only 1 subject in the 100 mg QD group had discontinuation due to drug-related pleural effusion compared with 35 subjects in the 70 mg BID group. In subjects with advanced phase CML or Ph+ ALL, drug-related pleural effusion accounted for discontinuation in 33 of the 1032 subjects. Six subjects in the 140 mg QD group had discontinuation due to drug-related pleural effusion compared with 27 subjects in the 70 mg BID group.

Overall, 59% (1287/2182) of subjects across all disease phases reported SAEs (any grade). Drug-related SAEs were reported in 53% (681/2182) of subjects. In subjects with chronic phase CML, notable common drug-related AEs included dyspnea, pleural effusion, congestive heart failure, febrile neutropenia, and thrombocytopenia. In most cases, a lower proportion of subjects in the 100 mg QD group reported drug-related SAEs than subjects in the 70 mg BID or other dose groups. In subjects with advanced phase CML or Ph+ ALL, notable common drug-related AEs included dyspnea, pleural effusion, diarrhea, and hematological toxicities. In most cases, there was little difference in these SAEs between the 140 mg QD and 70 mg BID groups. Rates of severe drug-related pleural effusion were lower in the 140 mg QD group (3%) vs. the 70 mg BID (6%).

The most frequently reported AEs are presented in Table 2.

Table 2: Very Common and Common AEs Reported in Subjects in Clinical Studies

	All Subjects (N= 2182) Percent (%) of Subjects	
	All Grades	**Grades 3/4**
Nervous system disorders *Very common*: headache	25	1

Table 2: Very Common and Common AEs Reported in Subjects in Clinical Studies

	All Subjects (N= 2182) Percent (%) of Subjects	
	All Grades	**Grades 3/4**
Common: neuropathy (including peripheral neuropathy)	7	<1
Dizziness	5	<1
dysgeusia	2	0
Somnolence	2	<1
Respiratory, thoracic and mediastinal disorders *Very common*: pleural effusion	27	7
Dyspnea	24	5
Cough	10	<1
Common: pulmonary edema	2	<1
lung infiltration	2	<1
pneumonitis	2	<1
pulmonary hypertension	1	<1
Gastrointestinal disorders *Very common*: diarrhea	33	4
Nausea	23	1
Vomiting	13	1
abdominal pain	11	<1
Common: gastrointestinal bleeding	8	4
mucosal inflammation (including mucositis/stomatitis)	7	<1
Dyspepsia	6	0
abdominal distension	5	0
Constipation	5	<1
gastritis	2	<1
colitis (including neutropenic colitis),	2	<1
oral soft tissue disorder	2	0
Skin and subcutaneous tissue disorders *Very common*: skin rasha	23	<1
Common: pruritus	7	<1
acne	5	<1
alopecia	5	0
dry skin	3	0
Hyperhidrosis	2	0
urticaria	1	<1

Table 2: Very Common and Common AEs Reported in Subjects in Clinical Studies

	All Subjects (N= 2182) Percent (%) of Subjects	
	All Grades	**Grades 3/4**
dermatitis (including eczema)	1	0
Musculoskeletal and connective tissue disorders *Very common*: musculoskeletal pain	15	1
Common: arthralgia	9	<1
Myalgia	8	<1
muscle inflammation	3	<1
muscular weakness	1	<1
musculoskeletal stiffness	1	0
Metabolism and nutrition disorders *Common*: anorexia	9	<1
appetite disturbances	2	<1
Hyperuricemia	1	<1
Infections and infestations *Very Common*: infection (including bacterial, viral, fungal, nonspecific)	11	3
Common: pneumonia (including bacterial, viral, fungal)	5	3
upper respiratory tract infection/inflammation	5	<1
herpes viral infection	1	<1
enterocolitis infection	1	<1
sepsis (including fatal outcome)	1	<1
Cardiac Disorders *Common*: pericardial effusion	5	1
arrhythmia (including tachycardia)	3	<1
congestive heart failure/cardiac dysfunction	3	2
palpitations	2	0
Vascular disorders *Very common:* hemorrhage	16	2
Common: flushing	4	0
hypertension	2	<1
Blood and lymphatic system disorders *Common:* febrile neutropenia,	5	5
pancytopenia	1	<1
General disorders and administration site conditions *Very common*: fatigue	23	2
superficial edema	22	1

Table 2: Very Common and Common AEs Reported in Subjects in Clinical Studies

	All Subjects (N= 2182) Percent (%) of Subjects	
	All Grades	**Grades 3/4**
pyrexia	14	1
Common: asthenia	9	<1
pain	8	<1
chest pain	6	<1
generalized edema	4	<1
chills	3	<1
Psychiatric disorders *Common:* insomnia	2	0
depression	2	<1
Eye disorders *Common:* visual disorder (including visual disturbance, vision blurred, and visual acuity reduced)	2	<1
dry eye	1	<1
Ear and labyrinth disorders *Common:* tinnitus	1	0
Investigations *Common*: weight increased	5	<1
weight decreased	5	<1
Injury, poisoning, and procedural complications *Common:* contusion	2	<1

Source: SPRYCEL® (dasatinib) BMS-354825, Bristol-Myers Squibb Investigator Brochure, Version 8, 20083

a. Includes drug eruption, erythema, exfoliative rash, fungal rash, maculo-papular rash, pustular rash, vesicular rash, skin exfoliation, and urticaria vesiculosa.

b. Includes ventricular dysfunction, cardiac failure chronic, cardiac failure congestive, cardiomyopathy, congestive cardiomyopathy, diastolic dysfunction, ejection fraction decreased, and ventricular failure.

c. Excludes gastrointestinal bleeding and CNS bleeding; These adverse drug reactions are reported under the gastrointestinal disorders system organ class and the nervous system disorders system organ class, respectively.

d. Includes auricular swelling, eye edema, face edema, gravitational edema, orbital edema, periorbital edema, scrotal edema, and tongue edema.

2.3.2 Experience in Phase 2 Breast Cancer Studies

Drug-related AEs in ≥ 25% of the subjects in the completed Phase 2 studies in breast cancer are given in Table 3. An additional Phase II trial in breast cancer has recently been reported [111] with similar toxicity profile.

Table 3: Drug-Related Adverse Events in ≥ 25% Subjects for Phase 2 Breast Cancer Studies CA180059 and CA180088

Preferred Term	CA180059, n (%)		CA180088, n (%)	
	70 mg BID (N=21)	70 mg BID (N=21)	70 mg BID (N=44)	100 mg BID (N=23)
Nausea	14 (67)	10 (44)	15 (34)	8 (35)
Fatigue	14 (67)	10 (44)	15 (34)	3 (13)
Diarrhea	7 (33)	12 (52)	23 (52)	10 (44)
Rash	5 (24)	11 (48)	10 (23)	9 (39)
Asthenia	0 (0)	0 (0)	13 (30)	9 (39)
Dyspnea	6 (29)	11 (48)	10 (23)	9 (39)
Pleural effusion	7 (33)	9 (39)	12 (27)	9 (39)
Anorexia	3 (14)	9 (39)	0 (0)	0 (0)
Headache	4 (19)	8 (35)	15 (34)	9 (39)
Cough	5 (24)	7 (30)	0 (0)	0 (0)
Abdominal pain	2 (10)	7 (30)	13 (30)	5 (22)
Vomiting	6 (29)	7 (30)	8 (18)	8 (35)
Flushing	6 (29)	3 (13)	0 (0)	0 (0)

2.3.3 Experience in Phase II Prostate cancer studies

The activity of dasatinib has been demonstrated in two Phase II trials of castrate-resistant prostate cancer. In the first Phase II, 47 men were treated with 100 mg or 70 mg twice daily. Dasatinib was generally well tolerated and treatment-related adverse events were moderate. Lack of progression was achieved in 43% of patients at week 12. [112] Another trial evaluated the combination of dasatinib with docetaxel in this population. [113] Finally, a trial performed in castrate-resistant prostate cancer using dasatinib 100 mg daily dosing was found to be safe and well-tolerated, with fewer adverse effects compared to the previous twice daily dosing trial. Moreover, essentially equivalent clinical activity was observed. [114]

Laboratory Abnormalities

Myelosuppression was commonly reported in all patient populations. The frequency of Grade 3 or 4 neutropenia, thrombocytopenia, and anemia was higher in patients with advanced CML or Ph+ ALL than in chronic phase CML. Myelosuppression was reported in patients with normal baseline laboratory values as well as in patients with pre-existing laboratory abnormalities (Table 4).

In patients who experienced severe myelosuppression, recovery generally occurred following dose interruption and/or reduction; permanent discontinuation of treatment occurred in 1% of patients. Grade 3 or 4 elevations of transaminases or bilirubin and Grade 3 or 4 hypocalcemia and hypophosphatemia were reported in patients with all phases of CML but were reported with an increased frequency in patients with myeloid or lymphoid blast CML and Ph+ ALL. Elevations in transaminases or bilirubin were usually managed with dose reduction or interruption. Patients developing Grade 3 or 4 hypocalcemia during the course of dasatinib therapy often had recovery with oral calcium supplementation (Table 4).

Table 4: CTC Grades 3/4 Laboratory Abnormalities in CML and Ph+ ALL

	Chronic (n=488)	Accelerated (n=186)	Blast Phase (n=132)	Ph+ ALL (n=105)
	Percent (%) of Patients			
Hematology Parameters				
Neutropenia	49	74	83	81
Thrombocytopenia	48	83	82	83
Anemia	18	70	70	51
Biochemistry Parameters				
Hypophosphatemia	11	13	23	21
Hypocalcemia	2	9	20	15
Elevated SGPT (ALT)	1	4	7	11
Elevated SGOT (AST)	1	2	5	8
Elevated Bilirubin	<1	1	5	8
Elevated Creatinine	0	2	1	1

CTC grades: neutropenia (Grade 3 ≥0.5–1.0 × 10^9/L, Grade 4 <0.5 × 10^9/L); thrombocytopenia (Grade 3 ≥10–50 × 10^9/L, Grade 4 <10 × 10^9/L); anemia (hemoglobin ≥65–80 g/L, Grade 4 <65 g/L); elevated creatinine (Grade 3 >3–6 × upper limit normal range (ULN), Grade 4 >6 × ULN); elevated bilirubin (Grade 3 >3–10 × ULN, Grade 4 >10 × ULN); elevated SGOT or SGPT (Grade 3 >5–20 × ULN, Grade 4 >20 × ULN); hypocalcemia (Grade 3 <7.0–6.0 mg/dL, Grade 4 <6.0 mg/dL); hypophosphatemia (Grade 3 <2.0–1.0 mg/dL, Grade 4 <1.0 mg/dL).

2.4 Anticipated Adverse Events

2.4.1 Myelosuppression

Treatment with dasatinib is associated with severe (NCI CTCAE Grade 3 or 4) thrombocytopenia, neutropenia, and anemia. Their occurrence is more frequent in patients with advanced CML or Ph+ ALL than in chronic phase CML. Complete blood counts should be

performed weekly for the first 2 months and then monthly thereafter, or as clinically indicated. Myelosuppression was generally reversible and usually managed by withholding dasatinib temporarily or dose reduction. In a Phase 3 dose-optimization study in patients with chronic phase CML, Grade 3 or 4 myelosuppression was reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily.

2.4.2 Bleeding Related Events

Severe CNS hemorrhages, including fatalities, occurred in $\leq$ 1% of patients receiving dasatinib. Severe gastrointestinal hemorrhage occurred in 4% of patients and generally required treatment interruptions and transfusions. Other cases of severe hemorrhage occurred in 2% of patients. Most bleeding events were associated with severe thrombocytopenia. (Incidences in this paragraph reflect drug-related adverse reactions based on investigator's attribution.)

Patients were excluded from participation in dasatinib clinical studies if they took medications that inhibit platelet function or anticoagulants. In some trials, the use of anticoagulants, aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) was allowed concurrently with dasatinib if the platelet count was 50,000 to 75,000. Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants.

2.4.3 Fluid Retention

Dasatinib is associated with fluid retention. In all clinical studies, severe fluid retention was reported in 10% of patients, including pleural and pericardial effusion reported in 7% and 1% of patients, respectively. Severe ascites and generalized edema were each reported in <1% of patients. Severe pulmonary edema was reported in 1% of patients. Patients who develop symptoms suggestive of pleural effusion such as dyspnea or dry cough should be evaluated by chest X-ray. Severe pleural effusion may require thoracentesis and oxygen therapy. Fluid retention events were typically managed by supportive care measures that include diuretics or short courses of steroids. (Incidences in this paragraph reflect drug-related adverse reactions based on investigator's attribution).

In the Phase 3 dose-optimization study in patients with chronic phase CML, fluid retention events were reported less frequently in patients treated with 100 mg once daily than in patients treated with 70 mg twice daily.

2.4.4 QT Prolongation

A comprehensive evaluation of data from Phase 2 studies (N = 865) examined the possible effect of dasatinib on ECG parameters, particularly the QTc interval. The mean QTc interval changes from baseline using Fridericia's method (QTcF) were 4 to 6 msec; the upper 95% confidence intervals for all mean changes from baseline were < 7 msec. On-study, a total of 5 subjects (<1%) reported a QTcF >500 msec; 1 of these 5 subjects reported a QTcF >500 msec on both Day 1 and Day 8. No events of Torsade de pointes were reported.

Nine of the 1150 subjects with chronic phase CML had QTc prolongation reported as an adverse event. Of these 9 subjects, 7 were considered related to drug. None of the 9 subjects who reported QTc prolongation were from the 100 mg QD group compared with 8 subjects from the 70 mg BID group. Ten of the 1032 subjects with advanced disease had QTc prolongation reported as an adverse event. Of these 10 subjects, 7 were considered drug-related. All 10 of the subjects who reported QTc prolongation were from the 70 mg BID group. Overall, of the 2182 subjects treated with dasatinib, 21 (1%) subjects across the studies reported a QTcF >500 msec .

2.4.5 Overall Risk/Benefit Assessment

The clinical studies discussed in Section 1.2 indicate that the safety profile for dasatinib in solid tumor subjects has been similar to that in chronic phase CML subjects with the exception of severe myelosuppression, which has not been observed in solid tumor subjects and is considered related to efficacy against the leukemia as noted above, and severe bleeding which is secondary to thrombocytopenia in most instances.

2.5 Phase I Experience in Solid Tumors

In a Phase I study (CA180003) conducted by Bristol Myers Squibb (BMS), dasatinib was administered on a BID schedule to 42 subjects with refractory solid tumor. To date, doses up to 160 mg BID on a 5-day on/2-day off schedule have been administered. [115]. [116]

No severe clinical toxicity has been encountered. Gastrointestinal symptoms were reported in most subjects, fatigue was reported in 17 subjects (40%) and rash in 10 subjects (24%). Edema, lethargy and headache were uncommon, and appear to be dose-related. Grade 3 asymptomatic hypocalcemia was considered dose-limiting in one subject, Grade 2 rash was considered dose-limiting in two other subjects, and Grade 2 nausea and vomiting (with dysarthria, lightheadedness and lethargy in a 49 kg subject taking concurrent diazepam) was considered dose-limiting in one subject.

In another Phase I study (CA180021), dasatinib was administered on a QD schedule to 24 subjects at doses up to 180 mg. Pleural effusions were observed in three subjects at the 180 mg dose level (one with pneumonia and two with malignant effusion). A dose of 250 mg QD is currently under consideration. Hypocalcemia, GI symptoms and skin rash have been mild and infrequent. [117] [118]

To date, the safety profile in solid tumor subjects has been similar to that in CP CML subjects with the exception of severe myelosuppression, which has not been observed in solid tumor subjects and is considered related to efficacy against the leukemia as noted above, and severe bleeding which is secondary to thrombocytopenia in most instances. [119, 120]

2.6 Justification for Once-Daily Dosing

Dasatinib was initially developed and approved for CML at a dose of 70 mg twice daily. Later post-registration studies have shown equivalent clinical efficacy in CML using once daily dosing schedules. This daily schedule has received a compendia listing and is generally favored in both academic and community practice for CML and ALL. This Phase I study will use once-daily dosing of dasatinib because it is expected to have equivalent clinical efficacy with fewer high-grade adverse effects., [120] The favorable profile of once daily dosing has been observed in other Phase II trials of advanced solid tumors (See Phase II trials), and described in the next three sections.

2.6.1.1 Comparison of Schedule Pharmacokinetics:

Dasatinib undergoes extensive metabolism after ingestion, with an elimination half-life of less than 4 hours [121]. In chronic-phase CML, dasatinib administered as 140 mg once daily or 70 mg twice daily resulted in similar plasma steady-state average concentrations, but the plasma steady-state trough concentration (C_{min}) was lower with 140 mg once daily. C_{min} levels correlated with dasatinib adverse effects [122].

2.6.1.2 Comparison of Schedule Pharmacodynamics:

Although not directly relevant to SRC inhibition in NSCLC, no statistically significant difference in clinical outcomes using daily dosing vs. twice daily has been observed in BCR-ABL addicted hematologic disorders. Specifically, in advanced-phase CML, no statistically significant differences were observed in major (MHC) or complete cytogenetic response (CCR), major (MaHR) or complete hematologic response (CHR), overall hematologic response or overall survival. In

Ph+ ALL, major cytogenetic response was more frequent (70%) in the once-daily group than in the twice-daily group (52%; P = 0.120). The same trends were observed in other indices (CHR 50% vs. 38%; MaHR 55% vs. 43%, and OHR 66% vs. 52%) [123].

In chronic-phase CML, dasatinib daily had equivalent outcomes using 1:1:1:1 randomization of 140 daily, 100 mg daily, 70 mg BID, and 50 mg BID [124]. The primary objective was achieved: imatinib-resistant patients receiving once-daily therapy attained a MCyR rate that was noninferior to the twice-daily schedule (once daily, 52% [95% CI, 45.4% to 58.2%]; twice daily, 49% [95% CI, 42.7% to 55.4%]; treatment difference, 2.8% [95% CI, –6.0% to 11.6%]). The main secondary objective was also achieved: the 100-mg total daily dose was noninferior to the 140-mg total daily dose among imatinib-resistant patients (100 mg, 50% [95% CI, 43.6% to 56.4%]; 140 mg, 51% [95% CI, 44.4% to 57.2%]; treatment difference, –0.8% [95% CI, –9.6% to 8.0%]).

Moreover, there is evidence that tyrosine kinases accumulate in solid tumors, including dasatinib-like compounds. For example, radiolabeled dasatinib accumulates in the tumors of mouse prostate cancers [125]. This indicates that achieving therapeutic plasma levels for over 24 hours may not be required for potent SRC kinase inhibition.

2.6.1.3 Comparison of Schedule Toxicity:

Studies in ALL and accelerated-phase CML have shown less toxicity using 140 mg rather than 70 mg BID dosing. In particular, there is less incidence of pleural effusions in both ALL (all grades, 18% versus 32%, NS) and AP CML (all grades 20% vs. 39%, P <0.001) [126] [123]. In AP CML, there were also fewer dose reductions (38% vs. 50%) and interruptions (64% vs. 74%) required in the once daily group. Pleural effusions were manageable and led to treatment discontinuation in only 4% (once-daily group) and 9% (twice-daily group) of patients. Pericardial effusions occurred slightly more frequently in the twice-daily group (once-daily vs. twice-daily: all grades, 3% vs. 7%; grade 3 to 4, 1% vs. 3%). The incidence of congestive heart failure or cardiac dysfunction was 0% and 3% in the once- and twice-daily groups, respectively.

In chronic-phase CML, generally fewer adverse effects were observed in 100 mg vs. 70 mg BID. Specifically, there were fewer grade 3-4 AEs (30% vs. 48%; P = .001), particularly grade 3 to 4 thrombocytopenia (22% vs. 37%; P = .004), and

less pleural effusions (of any grade) (7% vs. 16%; P = .024). However, the differences in AEs between 140 mg daily and 70 mg bid were essentially identical [124].

A meta-analysis of CML data concluded that bid dosing may be a risk factor for pleural effusion [127]. These reports prompted a Phase II trial with dasatinib in NSCLC to change their dosing from 70 mg bid to 100 mg daily [128].

Indeed, an original Phase II single-agent dasatinib in NSCLC reported a high rate of pleural effusion with 100 mg BID, causing investigators to perform an amendment to 70 mg BID [129]. Another Phase I study combined with erlotinib in NSCLC observed a higher incidence of several adverse effects including dyspnea with 70 mg BID cohort compared to 140 mg BID cohort [130]. Therefore, it is rationale and ethnical to perform trials in solid tumors using 100 mg to 140 mg once daily, rather than 70 mg twice daily, dosing schema.

3 AFATINIB DRUG PROFILE

BIBW 2992 (afatinib) is a highly selective and potent low molecular weight, irreversible inhibitor of the erbB-family of tyrosine kinase receptors EGFR (erbB1 / HER1), HER 2 (erbB2), and HER 4 (erbB4). All references in this protocol concerning BIBW 2992 refer to the free base compound BIBW 2992 BS which is used as the oral formulation.

The potency of afatinib was determined in enzymatic assays using recombinant human wild-type EGFR (IC50 0.5 nM) and HER2 (IC50 14 nM). A panel of recombinant human kinases tested in parallel was not inhibited, demonstrating the high target specificity of afatinib. Molecular modeling revealed that afatinib binds covalently and with high affinity to Cys773 within the catalytic cleft of the ATP-binding pocket of the EGF receptor. It has been reported that this specific molecular interaction results in irreversible inhibition of the EGFR tyrosine kinase domain.

The efficacy and potency of afatinib was demonstrated in vitro in receptor phosphorylation and cell proliferation assays in various human cancer cell models. The anti-proliferative effects observed with afatinib compare favorably to activity data published for gefitinib in the same NSCLC cell models. In addition, afatinib suppressed EGFR phosphorylation and clonogenic growth in the gefitinib resistant NCI-H1975 model, suggesting that tumor cells harboring the T790M EGFR TKI resistance mutation remain sensitive to this irreversible EGFR small molecule inhibitor (Table 5).

Cell line	Origin	EGFR status	BIBW 2992 IC_{50}		Gefitinib IC_{50}	
			EGFR-phosphorylation[1]	Proliferation *in vitro*[2]	EGFR-phosphorylation[1]	Proliferation *in vitro*[3]
NCI-H1666	NSCLC	Wild-type EGFR	7 nM	16 nM	100 nM	4 µM
NCI-H3255	NSCLC	L858R-mutation	6 nM	0.7 nM	50 nM	63 nM
NCI-H1975	NSCLC	L858R-mutation T790-resistance mutation	93 nM	99 nM	resistant	resistant

1 EGF induced auto-phosphorylation of EGFR
2 clonogenic anchorage-independent soft agar assay
3 MTS assay (R06-1388)

Table 5: In vitro activity of afatinib (BIBW 2992) in human tumor cell models expressing wild-type or mutated EGFR

The in vivo activity of afatinib against EGFR was investigated in an A431 subcutaneous xenograft model. Daily oral treatment with afatinib at doses of 20 mg/kg resulted in an almost complete inhibition of tumor growth over a period of 25 days. Similar anti-tumor activity was observed in NCI-N87 tumor bearing mice treated with afatinib at similar concentrations. In these in vivo studies, afatinib plasma concentrations of 80-285 nM corresponding to an AUC 0-24 of 589-3198 nM*h were required for anti-tumor activity. All afatinib doses shown to be effective in mouse xenograft models were well-tolerated.

3.1 Pharmacology and Toxicology Profile

The absolute bioavailability of afatinib after oral ingestion was 45% in rats, with a median t-max reached after 4 hours and a terminal half-life (t1/2) of 4.5 hours. In rats, the exposure was dose proportional, and no gender-related effects or compound accumulation was observed. Afatinib is primarily excreted via the feces. No relevant inhibition of cytochrome P450 isoenzymes was found. In vitro afatinib is however a CYP3A4 substrate. Because this is not considered a dominant metabolic pathway, in vivo drug-drug interactions with CYP3A4 inducers or inhibitors are not expected.

In vivo afatinib was metabolized only to a minor extent, and the metabolism was governed by adduct formation to proteins or nucleophilic small molecules. It was found that metabolism is of subordinate role for afatinib and that enzyme-catalyzed metabolic reactions play a negligible role for the metabolism of afatinib in vivo. Only approximately 2% of the dose was metabolized by FMO3 in vivo. The CYP3A4-dependent N-demethylation was even too low to be quantitatively detected in human volunteers. Therefore, intrinsic (e.g., genetic predisposition)

or extrinsic (e.g., by co-medications) effects on the activity of FMO3 or CYP3A4 in vivo are expected to be of little, if any, relevance for the pharmacokinetics of afatinib.

The human ADME data confirmed the results of the preclinical [14C] ADME studies and all metabolites of the human [14C] ADME study were observed in the rat or the minipig.

In acute toxicology studies, oral administration of single doses in rats and mice indicated a low acute toxic potential of afatinib. Changes in renal and hepatic function occurred only at doses that were 10- to 30-fold above the levels required for antitumor activity. afatinib had effects on gastrointestinal function that were dose-dependent and in high doses, leading to profound inhibition. No acute toxic effects on the central nervous system were detected.

In oral repeated dose studies for up to 26 weeks in rats and minipigs, the main target organs were the gastrointestinal tract (rats and minipigs), kidneys (rat), and the skin (rats). In the gastrointestinal tract, increasing systemic exposure was associated with dose-dependent atrophy of the epithelium and concomitant focal erosions/ulcerations in the stomach of rats and minipigs. Clinically, this resulted in diarrhea in both species and fecal occult blood in a single minipig. In rat kidneys papillary necrosis and dilated tubules were found. Similar pathologic findings, i.e., papillary necrosis in rats and dogs have been described previously for the EGFR-small molecule inhibitor gefitinib. However, nephrotoxicity has not been reported as a side-effect of gefitinib therapy in humans. A secondary pathophysiologic effect on renal function in afatinib-treated animals due to diarrhea-induced dehydration and emaciation has to also be considered. Cutaneous alterations, i.e., epithelial atrophy, were observed in rats. However, afatinib is not irritating to intact skin in albino rabbits, and the effects observed in rats are most likely related to the specific pharmacodynamic mechanism of EGFR inhibition. A variety of organs including the aerodigestive tract and reproductive organs were affected by epithelial atrophy. These atrophic changes were not severe and fully reversed during a 2-week recovery period. Minor cardiovascular effects (increased blood pressure and heart rate) and a dose-dependent decrease of QT time in the electrocardiogram (ECG) occurred in afatinib-treated minipigs. These data do not indicate a risk for QT-prolongation related arrhythmia. BIBW 2992 had no pro-arrhythmic potential, as determined by the effects on HERG-mediated potassium current or on guinea pig papillary muscle action potential configuration. BIBW 2992 demonstrated mutagenic potential in bacteria but had no genotoxic potential in vivo even at highly toxic/lethal doses in animals. Because of its specific pharmacodynamic mechanism of action, BIBW 2992 is potentially embryo/ fetotoxic and/or teratogenic.

3.2 Clinical Experience: Afatinib

3.2.1 Overview

At the time of 15 Jan 2013, 4634 patients had been exposed to afatinib in various Phase I-III trials. A summary of Phase I, II trials is given in tables below. Afatinib was administered to 100 healthy volunteers in an ADME study (1200.25), two bioavailability trials (1200.35 and 1200.80), and a drug-drug interaction study (1200.79).

More than 150 patients have been treated in Phase I monotherapy trials. A total of 409 patients were treated in Phase I or II combination trials, including four trials with the combination of BIBW 2992 and BIBF 1120. Phase II trials of afatinib have focused on non-small cell lung cancer (NSCLC), breast cancer, head and neck squamous cell carcinoma (HNSCC), and glioma. In Phase III trials, more than 2900 patients have been treated (either with afatinib or control treatment). The Phase III trial 1200.32 led to FDA-approval of afatinib in an orphan designation for first-line therapy of EGFR-mutant NSCLC on July 12, 2013.

In addition to the Phase III trials in NSCLC: 1, two more Phase III trials have begun patient enrollment. Phase III Trial 1200.34 in NSCLC is similar to 1200.32: it is being conducted in different countries and uses a different comparator chemotherapy regimen (cisplatin-gemcitabine as opposed to cisplatin-pemetrexed). Study 1200.75 is a randomized Phase III trial in advanced breast cancer in patients pre-treated with trastuzumab comparing BIBW 2992 plus vinorelbine versus trastuzumab plus vinorelbine.

Table 6: Early phase development of BIBW 2992. Adapted from [142]

Phase	Description	Primary end point	Ref.
Preclinical	*In vitro* and *in vivo* analysis of BIBW 2992	ErbB family inhibition	[131]
Preclinical	L858R and T790M transgenic mice	Tumor shrinkage	[132]
Preclinical	Combination with rapamycin in HER2-mutant mice	Tumor shrinkage	[133]
I	Dose-escalation study, once daily for 14 days. 10–100 mg	MTD	[134]
I	Dose-escalation study, once daily for 21 days. 10–65 mg	MTD	[135]
I	Dose-escalation study, once daily. Continuous. 10–50 mg	MTD	[136]
I	Dose-escalation study, once daily. Continuous. 10–60 mg	MTD	[137]
I	Dose-escalation study. BIBW 2992 (10–30 mg) every 6, 13 or 20 days in combination with docetaxel (60 or75 mg/m^2)	MTD	[138]
I	Dose-escalation study. BIBW 2992 (10–160 mg) 3 days post- docetaxel (65 or 75 mg/m^2)	MTD	[139]
I	Continuous study. BIBW 2992 (20–50 mg) plus paclitaxel and bevacizumab	MTD	[140]
II	*EGFR* mutations/gene amplification (FISH-positive)	ORR (CR and PR)	[141]

3.2.2 Dose Selection for Single-Agent Trials of Afatinib

The most up to date trial and safety information can be found in the current version of the Investigator Brochure. Afatinib showed moderately fast absorption with median tmax values between 1 h to 6 h after administration. The gMean terminal half-life (t1/2) of afatinib mainly ranged between 13 h to 57 h. In general, the maximum blood concentration (Cmax) and the integral of the concentration time curve (AUC) of afatinib increased in a dose-proportional way.

The maximum tolerated dose (MTD) of afatinib was identified as 50 mg once daily in phase I continuous dosing monotherapy trials. The 50 mg dose is currently used in the Phase IIb/III trial 1200.23 in NSCLC patients progressing on erlotinib or gefitinib, as maximum EGFR inhibition is required in this last-line population enriched for the presence of resistance mutations. A starting dose of 30 mg is expected to be sufficient. In phase I clinical trials of afatinib, durable responses (>20 months) were seen at daily doses of 40 mg and less. In this trial, a starting dose of 30 mg will be used in order to optimize the efficacy/toxicity balance with dasatinib.

3.2.3 Adverse Events with Afatinib

The Adverse Events (AEs) observed to date in phase I and phase II trials are consistent with those reported for other EGFR tyrosine kinase inhibitors (dose-dependent diarrhea and skin-related adverse events including rash and acne). Other AEs were in the expected range for patients with advanced cancer disease. In the BIBW 2992 phase I monotherapy trials, the most frequent drug-related adverse events were associated with gastrointestinal disorders (diarrhea, nausea, vomiting, stomatitis), skin and subcutaneous tissue disorders (rash, dry skin, pruritus, acneiform rash, and acne), general disorders and administration site conditions (fatigue, mucosal inflammation), respiratory disorders (epistaxis, typically grade 1), and metabolism and nutritional disorders (anorexia, dehydration).

Diarrhea is the single most often reported gastrointestinal AE. An increased incidence of diarrhea grade 3 has been observed in phase II monotherapy trials. Dose reduction is crucial to reduce the severity of diarrhea and its potential complications such as dehydration, leading to serum electrolyte changes (hyponatremia, hypokalemia and hypomagnesaemia) and/or renal impairment. Nausea and vomiting are the other commonly reported gastrointestinal adverse events and can be generally managed successfully with the use of antiemetics.

Skin-related adverse events present in a number of forms, i.e., rash (including erythematous, maculo-papular, papular, etc.), acne, dermatitis acneiform, dry skin, skin reaction, and pruritis. Folliculitis as well as nail changes (including paronychia) are other reported manifestations of skin-related adverse events with BIBW 2992. Early and adequate management of skin-related adverse events can reduce the frequency and the severity of them.

Further AEs include oral discomfort (stomatitis, mouth ulceration, oral pain, dry mouth), soft tissue disorder, and mucosal inflammation. Conjunctivitis and rhinorrhea as a result of inflammation in the mucosal membranes have been reported. Mucosal and skin dryness can lead to epistaxis, which has always been observed at CTCAE Grade 1. Anorexia, fatigue and asthenia are also frequently observed.

3.2.4 Clinical Efficacy

The FDA approval of afatinib was based on the demonstration of improved progression-free survival (PFS) in a multi-center, international, open-label, randomized (2:1) trial, called LUX-lung 3. This trial enrolled 345 patients with metastatic NSCLC whose tumors tested positive for EGFR mutations. Patients were randomized to receive afatinib 40 mg orally once daily (n=230) or pemetrexed/cisplatin (n=115). Randomization was stratified according to EGFR mutation status (exon 19 deletion vs. exon 21 L858R vs. 'other') and race (Asian vs. non-Asian). The

major efficacy outcome was progression-free survival (PFS) as assessed by an independent review committee (IRC).

Of 345 patients enrolled, 65% were female, the median age was 61 years, 26% were Caucasian, and 72% were Asian. The majority of patients had a tumor sample with an EGFR mutation categorized as either exon 19 deletion (49%) or exon 21 (L858R) substitution (40%), while the remaining 11% had 'other' mutations.

A statistically significant prolongation of PFS determined by the IRC was demonstrated for patients assigned to the afatinib treatment arm [HR 0.58 (95% CI: 0.43, 0.78); $p < 0.001$, stratified log-rank test]. The median PFS was 11.1 months in the afatinib arm and 6.9 months in the chemotherapy arm. Objective response rates were 50.4% and 19.1% in the afatinib and chemotherapy arms, respectively. No statistically significant difference in overall survival between the two arms was demonstrated. In patients whose tumors have exon 19 deletions or exon 21 (L858R) substitution mutations, the median PFS was 13.6 months in the afatinib arm and 6.9 months in the chemotherapy arm.

Of 22 evaluable patients with NSCLC from phase I trials, there have been four partial responses (PRs) (3 confirmed and 1 unconfirmed PR) ranging in duration from 5 months to 24 months. All 4 patients were non-smoking Caucasians with adenocarcinoma of the lung. In two of these three patients with confirmed PR, EGFR sequencing has shown exon 19 in-frame deletions. These preliminary clinical efficacy data suggest that BIBW 2992 may be efficacious in patients with recurrent NSCLC harboring sensitizing EGFR mutations. The efficacy and safety of BIBW 2992 in NSCLC is being evaluated in a phase II trial. In this trial, patients with Stage IIIB/IV lung adenocarcinoma with EGFR mutation in exons 18-21 and failure of one line of systemic chemotherapy are treated with BIBW 2992 at a dose of 50 mg once daily until disease progression. Preliminary results of the first 24 patients (never-smoker: 14; ex-smoker: 8, and current smoker: 2) treated in the 2nd line setting have shown tumor size reductions in the majority of patients with disease control rate of 87.5% and overall response rate of 50% (CI: 30-70%). Diarrhea (83.3%) and skin-related adverse events (87.4%) have been the main side effects but have been manageable with appropriate dose interruption/reduction (P08-07355). This trial has now been amended to allow inclusion of chemotherapy-naïve patients.

A global, randomized, placebo controlled, double blind phase III trial (LUX-Lung 1) is one of several currently ongoing Phase III trials, and is assessing the efficacy of afatinib plus best supportive care (BSC) when compared to placebo plus BSC in non-small cell lung cancer patients failing erlotinib or gefitinib. Based on the favorable planned interim analysis of safety and efficacy described in the protocol, the independent DMC has allowed for continuation of

the trial. [143]. Additional Phase II/III trials are investigating afatinib in advanced non-small cell lung cancer, including EGFR wild-type and mutation positive populations (See Table 7).

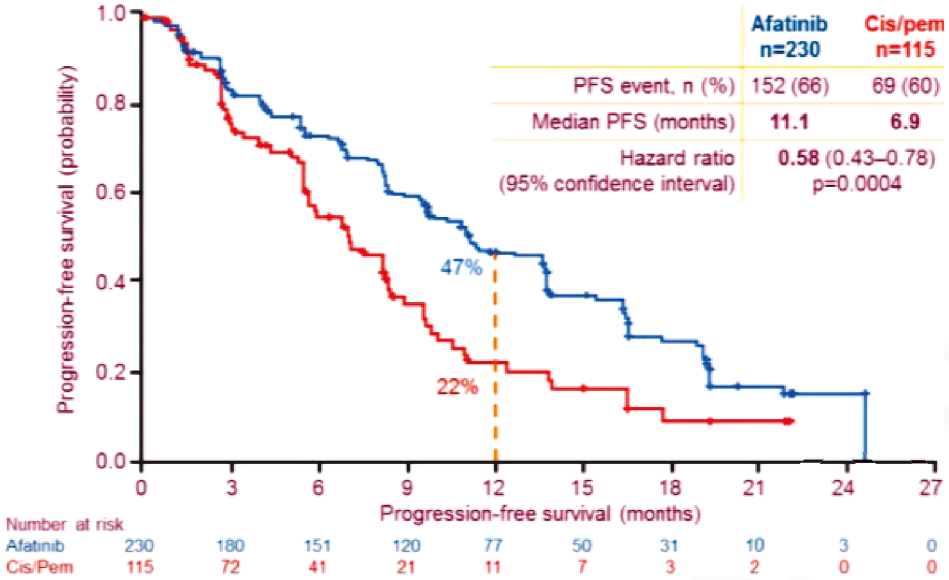


Figure: LUX-Lung 3 was a randomized, open-label, phase III trial which demonstrated superiority of BIBW 2992 (afatinib) over pemetrexed / cisplatin as first-line treatment for patients with advanced NSCLC harboring EGFR mutations. (J. Yang *et al.* J Clin Oncol 30, 2012 suppl; abstr LBA7500)

3.2.5 Pharmacokinetics

Preliminary data in clinical trials indicate that afatinib was absorbed moderately fast after oral administration, with median tmax values approximately 3 hours after drug administration. In general, afatinib gMean maximum plasma concentrations and exposure increased with increasing doses after a single dose and at steady state. However, moderate to high inter- and intra-individual differences in plasma concentrations were seen. Afatinib is highly distributed out of the blood and has a moderate to high clearance. The overall gMean terminal half-life at steady state was 37.2 hours in cancer patients and 27.5 to 33 hours in healthy volunteers after single dose administration. Steady state was reached no later than 8 days after the first administration. The major route of elimination of afatinib was via the feces. After food intake, a decreased systemic exposure was observed compared to administration of afatinib under fasted conditions. The PK characteristics in Caucasian cancer patients were comparable to those observed in Japanese cancer patients. The PK characteristics in healthy volunteers following single dose were comparable to those observed in cancer patients. In a population PK analysis in cancer patients, no statistically significant effect of the covariates alcohol consumption, smoking status, ethnic origin, cancer type, age, presence of liver metastases, hepatic impairment or liver function parameters alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (BIL), and total plasma protein was observed. Statistically significant, albeit small, increases were observed for concurrent food intake, low body weight, ECOG performance score (>1), low creatinine clearance, high lactate dehydrogenase levels,

and female gender. None of the individual covariate effects resulted in a fractional change in exposure of more than 25% relative to the exposure in a typical patient of the analyzed population.

At relatively high concentrations, afatinib is a substrate and inhibitor of P-gp in preclinical models. BI performed a Phase I trial (1200.79) at the 20 mg dose of afatinib, which was designed to determine the maximum effect of potent P-gp inhibition by ritonavir on the PK of afatinib. The study shows that the rate and extent of absorption of afatinib was increased by co-treatment with ritonavir. When taken in combination with ritonavir, the exposure to afatinib increased by 50.0%, 47.6%, and 38.5% for AUC0-∞, AUC0-tz and Cmax, respectively. Therefore, participants are excluded who are receiving concurrent P-gp inhibitors. (See Exclusion Criteria). Dasatinib is not a P-gp inhibitor. (BMS Investigator's brochure 2010).

3.2.6 Phase II/ III Trials

Table 7: Afatinib Trials in patients with NSCLC: Adapted from [142], [70]

Title	Phase	Trial status	Description	Patients	Primary	BIBW dose	Ref.
LUX−Lung 1	IIb/III	Completed	Chemo and EGFR TKI pretreated	585	OS	50 mg	[144]
LUX−Lung 4	I/II	Analysis ongoing	Chemo and EGFR TKI pretreated	90	PI: Safety PII: ORR	50 mg	[145]
LUX−Lung 5	III	Ongoing	BIBW 2992 beyond progression	900	PFS	50 mg	[70]
Trials in patients whose tumors harbor* EGFR *mutations (first-line)							
LUX−Lung 2	II	Analysis ongoing	*EGFR* mutation-positive 1st & 2nd -line)	129	ORR	40 or 50 mg	[146]
LUX−Lung 3	III	Completed	*EGFR* mutation-positive (1st line)	330	PFS	40 mg	[70]
LUX- Lung 6	III	Ongoing	*EGFR* mutation-positive (1st line)	330	PFS	40 mg	[70]

EGFR: EGF receptor; ORR: Objective response rate; OS: Overall survival; PFS: Progression-free survival; TKI: Tyrosine kinase inhibitor.

3.3 Afatinib – Combination-Specific Safety Measures.

In clinical studies, afatinib has been investigated with docetaxel at its commonly used dosing regimen (Trials 1200.6 and 1200.20). PK data from Trial 1200.6 suggest that there was no influence of 20 days administration of afatinib 20 mg on the PK of docetaxel (75 mg/m2). In addition, PK data from trial 1200.20 demonstrated that there was no clinically relevant

pharmacokinetic interaction observed between afatinib and docetaxel in the applied dosing schedule.

In Trial 1200.5 afatinib was administered once daily at a starting dose of 50 mg (which was reduced to 40 mg and later on to 30 mg per protocol amendments) over repeated 28-day treatment periods (TP) concomitantly with 2.5 mg daily letrozole. The PK data of this trial suggest that there was no influence of afatinib in the PK of letrozole and vice versa.

Since afatinib was found to be a P-gp substrate in vitro, BI performed a Phase I trial 1200.79 in healthy volunteers to assess the effects of the potent P-gp inhibitor ritonavir (dose 200 mg bid for 3 days) on the pharmacokinetics of afatinib (dose 20 mg). The study was designed to determine the maximum effect of P-gp inhibition on the PK of afatinib. The median tmax and terminal half-life of afatinib was not affected; however, the rate and extent of absorption of afatinib were increased by co-treatment with ritonavir. The relative bioavailability of afatinib when taken in combination with ritonavir (AUC0-∞, AUC0-tz and Cmax) increased to 150.0%, 147.6%, and 138.5%, respectively. The increase in rate and extent of afatinib upon co-administration of the P-gp and CYP 3A4 inhibitor ritonavir without apparent effect on median tmax, distribution, and elimination of afatinib point to an impact of ritonavir on P-gp-mediated transport processes during the absorption phase of afatinib. Since previous studies revealed that especially CYP3A4 enzyme-catalyzed metabolic reactions play a subordinate role for the metabolism of afatinib in vivo, the increase in the exposure of afatinib in the presence of ritonavir can be attributed most likely to inhibition of P-gp-mediated transport processes during the absorption phase of afatinib.

3.4 Conclusions From Pharmacokinetics in Humans

Afatinib exhibited at least biexponential disposition kinetics. After oral administration, median tmax values were at 3.00 hours for single dose as well as for steady state in cancer patients. Steady state was reached within 8 days after the first administration. A high apparent volume of distribution during the terminal phase and a moderate to high apparent total body clearance for afatinib has been calculated. The values obtained for total body clearance and volume of distribution should be interpreted with caution as the absolute bioavailability of afatinib in humans is yet unknown. The overall gMean terminal half-life at steady state was 37.2 hours in cancer patients and 27.5 to 33 hours in healthy volunteers after single dose administration. The overall gMean accumulation ratios for afatinib were 2.83 based on AUC and 2.14 based on Cmax. Moderate to high inter-patient variability in all afatinib PK parameters was detected. The PK characteristics in Caucasian cancer patients were comparable to those observed in

Japanese cancer patients. The PK characteristics in healthy volunteers following single dose were comparable to those observed in cancer patients. [136, 147]

Afatinib maximum plasma concentrations and exposure were found to increase more than dose proportionally in healthy volunteers (dose range 20 to 50 mg). Afatinib displayed a food effect with a decreased systemic exposure in the fed state compared to administration of afatinib under fasted conditions. The major route of elimination of total [14C] radioactivity was via the feces (85.4%). The contribution of renal excretion to the total clearance was low (4.29%). The overall recovery of [14C]-radioactivity was 89.5% and can therefore be regarded as complete mass balance. The coadministration of the potent P-gp inhibitor ritonavir increased the AUC0-∞, AUC0-tz and Cmax of afatinib to 150.0%, 147.6%, and 138.5%, respectively.

In a population PK analysis in cancer patients, no statistically significant effect of the covariates alcohol consumption, smoking status, ethnic origin, cancer type, age, presence of liver metastases, hepatic impairment or liver function parameters alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (BIL), and total plasma protein was observed. Statistically significant effects were observed for concurrent food intake, body weight, ECOG performance score, creatinine clearance, lactate dehydrogenase levels, and gender (small effect size).

Since afatinib is neither an inhibitor nor major substrate of CYP3A4, it is not expected to alter the pharmacokinetics of dasatinib. Since dasatinib is neither an inhibitor, inducer, or substrate of Pgp, it is not expected to alter the pharmacokinetics of afatinib.

3.5 Afatinib Formulation

The oral tablets are conventional, immediate-release tablets containing afatinib as the hydrochloride salt. The available dose strengths 20 mg, 30 mg, and 40 mg film-coated immediate release tablets are prepared in a common blend, with higher drug load per tablets.

A color scheme was established to distinguish between the doses and tablet sizes. The 40 mg film-coated tablets are light blue and of round, biconvex, bevel-edged shape with a diameter of approximately 8 mm and 10 mm, respectively. The film-coated 30 mg tablets are dark blue. The 30 mg film-coated tablets are as well round, biconvex, bevel-edged shape with a diameter of approximately 9 mm. The 20 mg film-coated tablets are light blue, round, biconvex, bevel-edged shape with a diameter of approximately 9 mm. All doses are either one-sided embossed with the Boehringer Ingelheim logo or double-sided embossed with the Boehringer Ingelheim logo on one side and a combination of the letter “T” with the dose strength on the other side. For trial tablet specifications, see Product Identification.

3.6 Dosage, Administration, and Storage

Afatinib will be self-administered in an open-label, unblinded manner to all patients enrolled in the study. During the treatment period, patients will receive single-agent Afatinib. Tablets should be taken at the same time each day with 200 mL of water at least 1 hour before or 2 hours after a meal. Patients who are unable to swallow tablets may dissolve the tablets in distilled water for administration.

Afatinib tablets will be supplied for clinical trials in white, high-density polyethylene (HDPE) bottles with child-resistant closures and should be stored at temperatures between 15°C and 25°C (59°F and 75°F). Based on 30 months stability data for the 20 mg and 50 mg dosage strength of the newly available film-coated tablet in HDPE bottles with desiccant at 25°C/60% RH, 30°C/75% RH, and 40°C/75% RH a preliminary shelf-life of 30 months has been assigned for all the dosage strengths in all climatic zones. Storage statements for climatic zones I/II will be “Do not store above 30°C”; for climatic zones III/IV, the statement will read “Do not store above 25°C.” For all climatic zones, statements will be “Store in the original package, in order to protect from light” and “Keep the bottle tightly closed, in order to protect from moisture.” (See Handling and Dispensing).

4 STUDY DESIGN

4.1 Overview

Patients with histologically proven unresectable stage IIIB /IV NSCLC, and an Eastern Cooperative Group (ECOG) performance status of 0-1 are eligible. With the exception of T790M mutation positive tumors, participants must have relapsed or been refractory to at least one line of prior systemic treatment (See Inclusion Criteria). Patients are required to have adequate hematologic, hepatic, and renal function and have relapsed after at least one prior chemotherapy regimen for recurrent or metastatic disease. Patients must give written informed consent. The Scientific Review Committee of the Moffitt Cancer Center and Institutional Review Board must approve the study (see Monitoring), which will be conducted in accordance with federal and institutional guidelines.

This is a dual-agent, open-label, phase I study. Eligible patients will be enrolled and treated according to the schema outline. Three patients will be treated per cohort for one cycle (28 days per cycle). If no DLTs are recorded, treatment will continue and three additional patients will be treated in the subsequent cohort. However, if a patient develops a DLT, another three

patients will be treated in this cohort for one cycle. If there are no more DLTs, dose escalation will continue. If more than one of three patients develops a DLT in any cohort, another three patients will be treated in the next lower dosage cohort. No intra-patient dose escalation will be permitted. Toxicity will be graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 4. (http://ctep.cancer.gov/forms/CTCAEv4.pdf).

The maximum tolerated dose (MTD) for this combined treatment will be defined as either:

(1) the highest dosage cohort in which six patients had been treated and there were less than two DLTs or,

(2) Afatinib at the highest tolerated dose investigated (40 mg PO daily) plus dasatinib at the highest tolerated dose investigated (cohort 3, 140 mg PO daily).

Then, twenty additional patients (total) will be treated in a Phase IB extension group at the MTD to confirm tolerability and determine the secondary endpoint of a preliminary clinical efficacy signal.

Tumor size will be assessed via computed tomography every 56 ± 7 days. Modified Response Evaluation Criteria in Solid Tumors (RECIST v 1.1) will be used to determine tumor response and disease progression. See Phase IA and Phase IB Study calendars.

5 SELECTION OF PATIENTS

5.1 Inclusion Criteria

[1] Pathologically or cytologically documented Stage IIIB/IV non-small cell lung cancer, or unresectable recurrent disease following locoregional treatment.

[2] *For Phase IB Extension Only*:

[2.1] Either or both of the following:

a) A tumor which harbors an activating Epidermal Growth Factor Receptor (EGFR) -mutation:

b) History of objective response, or stable disease for at least 6 months, after treatment with erlotinib, afatinib, or gefinitib.

[2.2] Either or both of the following:

a) Progression or recurrence of disease after receiving prior continuous gefinitib, afatinib, or erlotinib

b) A tumor known to harbor a *de novo* T790M mutation, which is known to confer EGFR TKI resistance.

[2.3] Participants are allowed to have received systemic chemotherapy or investigational therapy in the intervening period prior to trial enrollment

[3] Capable of giving written informed consent.

[4] Evaluable disease, as follows:

a) *For Phase IA Dose Escalation:* Have the presence of any evaluable disease, including bone metastases, effusion, or cystic metastases.

b) *For Phase IB Extension Only*: Have progressive and measurable disease as defined by the Response Evaluation Criteria in Solid Tumors (RECIST v 1.1).

[5] Reproductive potential must be either terminated (by surgery, radiation, or menopause) or attenuated by the use of an approved contraceptive method during and for 3 to 6 months following the study.

[6] Are at least 18 years of age.

[7] Patient agrees that IV bisphosphonates will be withheld during the first 8 weeks of dasatinib therapy due to risk of hypocalcemia.

[8] Have recovered from prior drug-related toxicity to Grade ≤ 1 CTCAE v4, within 21 days of initiation of on-study treatment.

[9] ECOG performance status of 0 or 1 at initial enrollment, as assessed by clinician or investigator.

5.2 Exclusion Criteria

[1] Have previously completed or withdrawn from this study or any other study investigating dasatinib. Prior treatment with other tyrosine kinases, including afatinib, is acceptable.

[2] Prior recent systemic or investigational therapy within 21 days of initiation of study treatment. An exception is that EGFR inhibitor may be continued up until 3 days of initiation of study treatment. [7]

[3] Women who are pregnant or breastfeeding. Women of child-bearing potential must have a negative pregnancy test (β-HCG test in urine or serum) prior to commencing study treatment.

[4] Patients with documented central nervous system or leptomeningeal metastasis (brain metastasis) at the time of study entry. Patients with prior brain metastasis may be considered if they have completed their treatment for brain metastasis and no longer require corticosteroids.

[5] Patients with disease progression in the CNS only.

[6] Serious concomitant disorder, including active bacterial, fungal, or viral infection, incompatible with the study (at the discretion of the principal investigator).

[7] Uncorrected severe electrolyte disorder, including severe potassium (<3.0 mEq/L) or magnesium (< 1.0 mEq/L) deficiency.

[8] Any gastrointestinal disorder with diarrhea as a major symptom, such as Crohn's, or pre-existing chronic diarrhea CTC Grade ≥ 2 of any etiology. Included are malabsorption disorders that in the opinion of the study physician may affect absorption of either afatinib or dasatinib.

[9] Prior major surgery or radiation therapy within 14 days of initiation of treatment.

[10] Electrocardiogram (ECG) abnormalities indicative of arrhythmia (at the discretion of the investigator).

[11] History or presence of clinically relevant cardiovascular abnormalities such as uncontrolled hypertension, congestive heart failure NYHA classification of 3, unstable angina or poorly controlled arrhythmia. Myocardial infarction within 6 months prior to enrollment.

[12] Baseline (< 1 month before treatment) cardiac left ventricular function with resting ejection fraction of less than 50% measured by multigated blood pool imaging of the heart (MUGA scan) or echocardiogram.

[13] Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, congenital long QT syndrome, or Torsades de pointes).

[14] Prolonged QTc interval on pre-entry electrocardiogram (> 470 msec for men and >480 msec for women per AHA/ACC 2011 scientific statement [148]).

[15] History of significant bleeding disorder unrelated to cancer, including diagnosed congenital bleeding disorders (e.g., von Willebrand's disease).

[16] Patients currently taking drugs that are generally accepted to have a high risk of causing Torsades de Pointes including: quinidine, procainamide, disopyramide, amiodarone, sotalol, ibutilide, dofetilide erythromycins, clarithromycin, chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide, cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine , ranozaline, and St. John's wort.

[17] Patients with pre-existing interstitial lung disease (ILD), or pericardial / pleural effusion of grade 2 or higher. Trace pericardial or pleural effusion is acceptable.

[18] Patients who require chronic oxygen therapy for chronic obstructive pulmonary disease or pleural effusions (malignant or benign).

[19] Patients requiring comedication with potent P-gp inhibitors (including cyclosporin, azithromycin, erythromycin, ketoconazole, itraconazole, quinidine, phenobarbital salt with quinidine, ritonavir, valspodar, verapamil) or inducers (including rifampicin).

[20] Known active hepatitis B infection, known active hepatitis C infection, or known HIV carrier.

[21] Known or suspected active drug or alcohol abuse.

[22] Known hypersensitivity to afatinib, dasatinib, or the excipients of any of the trial drugs.

[23] Laboratory exclusion criteria:

Absolute neutrophil count (ANC) < 1000 / mm3,

Platelet count < 100,000 / mm3, Serum creatinine ≥1.5 times the upper normal limit or calculated/measured creatinine Clearance ≤60 mL/min.
Total bilirubin ≥1.5 mg/dL (>26 mol/L, SI unit equivalent)

Aspartate amino transferase (AST) or Alanine amino transferase (ALT) ≥three times the upper limit of normal (if related to liver metastases ≥ five times the upper limit of normal)

6 TREATMENTS

6.1 Study Calendar – see Study Calendar section.

Notes:

- ***Physical examination*** includes complete vital signs: blood pressure, heart rate, respiration rate, temperature, height and weight, and ECOG performance status.
- A physician H&P must be performed within 14 days of enrollment.
- *CMP* = AST/ALT, alkaline phosphatase, total bilirubin, albumin, total protein, urea, creatinine, electrolytes, glucose, and calcium. Mag = magnesium.
- *B-HCG* in women of child-bearing potential.
- Plasma samples are for future exploratory retrospective studies only.
- Scans for baseline tumor evaluation should be performed within 28 days prior to enrollment.

- Response evaluation can be performed on Cycle 2, Day 22 ± 7 days; for patients continuing on study past cycle 2, assessments of response will occur every other cycle, Day 22 ± 7 days.

- LVEF assessment will continue every 3 months +/- 14 days while on afatinib therapy until one year (365 days) after initiation of study drug.

6.2 Treatments Administered

6.2.1 Screening Evaluation

Prior to the registration at study entry, all patients must have given written informed consent for the study and must have completed the pre-study evaluations. Patients must meet all of the eligibility requirements listed. The following is required as part of study screening:

Complete medical history including dates and description of initial diagnosis of NSCLC and documentation of any previous treatment for cancer (chemotherapy, surgery and/or radiation. Any on-going adverse events resulting from prior cancer therapies (radiotherapy, surgery, etc.) will be recorded (within 14 days before enrollment) using the NCI-CTCAE Version 4.0 (Expanded Common Toxicity Criteria (Version 4.0), as well as tumor-related signs and symptoms.

A complete physical examination is required (performed within 14 days prior to enrollment) including, but not limited to, vital signs, height, weight, ECOG Performance Status, and (if applicable) any observable tumor measurements.

Clinical laboratory testing: CBC with differential and platelet count, total protein, total serum bilirubin, alkaline phosphatase, AST (SGOT), ALT (SGPT), serum creatinine, serum electrolytes, serum glucose, serum urea, serum calcium, serum magnesium and serum albumin are required (performed within 14 days prior to enrollment). A pregnancy test for female patients of childbearing potential is required.

Serum magnesium is required due to potential risk of hypomagnesaemia from diarrhea.

A screening EKG is required. This should be performed within 28 days prior to enrollment.

A screening MUGA or ECHO is required. This should be performed within 28 days prior to enrollment. LVEF assessment will continue every 3 months +/- 14 days while on afatinib therapy until one year (365 days) after initiation of study drug.

Tumor evaluation: Appropriate clinical testing will be used to evaluate all known sites of malignant lesions, including CTs of the chest and upper abdomen including the adrenal glands; ultrasound; or radionuclide scans of the bones; and/or other radiographic studies

should be performed within 28 days prior to enrollment. To ensure comparability, the baseline scans and subsequent scans used to assess response must be performed using identical techniques. For Phase IB extension cohort, lesions must be measurable and evaluable by modified RECIST criteria.

6.2.2 Cycle 1

C1D1: Patients will have CBC, CMP, magnesium on day 1 before administration of study drug(s). Any residual toxicity from prior therapy for cancer will be recorded. Blood will be drawn for assessment of serum markers. The patient will begin afatinib at the appropriate cohort dose starting C1D1 on a daily basis. For Phase IB, the patient will begin afatinib and dasatinib concurrently.

C1D8: (Phase IA only) Toxicity of afatinib will be assessed. Patients start dasatinib. The patient will take afatinib and dasatinib tablets by mouth and will continue to take daily doses of afatinib and dasatinib per the dose escalation cohort on a daily basis.

C1D15: Patients will have interval EKG, CBC, and CMP. Toxicity of afatinib and dasatinib will be assessed. The patient will continue afatinib and dasatinib tablets by mouth and will continue to take daily doses of afatinib and dasatinib per the dose escalation cohort on a daily basis.

6.2.3 Cycle 2

C2D1: Patients will be seen by the treating physician and have complete H&P, CBC, and CMP. Blood will be drawn for assessment of serum markers. Toxicity of afatinib and dasatinib will be assessed. The patient will continue afatinib and dasatinib tablets in clinic by mouth and take daily doses of afatinib and dasatinib per the dose escalation cohort on a daily basis.

C2D22: Patients will undergo reevaluation for tumor measurements. This assessment can occur on C2D22 ±7 days. Response to treatment will be assessed per criteria as described in accompanying Evaluation of Response section.

6.2.4 Therapy Beyond Cycle 2

C3D1: Patients will be seen by the treating physician and have complete H&P, CBC, and CMP. A surveillance ECHO or MUGA will be performed, C3D1 +/- 7 days. The surveillance study should be the same modality as the original baseline study (ECHO or MUGA). Toxicity of afatinib and dasatinib will be assessed. Results on the tumor assessment and response to treatment will be assessed every two cycles after cycle 2 D22 evaluation. Patients who have

documented response or stable disease and have no DLT may continue on therapy. The patient will then take afatinib and dasatinib tablets in clinic by mouth and will continue to take daily doses of afatinib and dasatinib per the dose escalation cohort on a daily basis.

Patients continuing on therapy past two cycles will be seen by the treating physician every 4 weeks and will have complete physical examination, CBC, and CMP. Tumor measurement and response assessment will occur every 6-8 weeks as outlined above. Dasatinib and afatinib will be continued until progression of disease, unacceptable toxicity, patient request, or at discretion of the sponsor/ investigator.

6.2.5 End of Treatment visit (EOT)

Those that have documented progression and/or DLT or any other reason to come off of protocol will be removed from further therapy and reasons documented. A toxicity assessment and physical examination will be performed. If EOT visit is not possible, for example due to concurrent extramural inpatient hospitalization or death, these extenuating circumstances must be documented and reviewed with the primary investigator(s). See for Section: Withdrawal Criteria for additional reporting and participant withdrawal procedures. Also see section: Time Period Reporting Requirements. EOT visit should be completed within 21 days of cessation of study drug.

6.3 Dose Escalation Rules

6.3.1 Dose Escalation Schema

The dose escalation scheme is shown in Table 8, and the rules of dose escalation are shown below. These doses of dasatinib are based on Phase I data that are important for testing along with preclinical data suggesting response in cells [149]. The initial dose of afatinib in cohort 1 was chosen based on results from combination Phase I studies with taxanes and another small molecule inhibitor (See Phase I/II experience).

Table 8: Dose Escalation Schema

Phase I Cohort	BIBW2992 Begins Day #1	Dasatinib Begins Day #8
1.0	30 mg	100 mg
2.0	40 mg	100 mg
3.0	40 mg	140 mg

Number of Patients with DLT	Escalation Decision Rule
0/3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose. Three additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1/3	Enter at least 3 more patients at this dose level. • If 0/3 patients experience DLT, proceed to the next dose level. • If ≥1 suffers DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is will be the maximum tolerated dose (MTD). At least 6 patients must be entered at this dose. This will be the dose chosen for Phase IB.

Three patients are treated per cohort for one cycle (28 days per cycle). If no DLTs are recorded on the three patients in each cohort, treatment continues and three patients are treated in the subsequent cohort. However, if a patient develops a DLT, another three patients are treated in this cohort for one cycle. If there are no more DLTs, dose escalation continues. If more than one of three patients develops a DLT in any cohort, this dose is declared the maximally administered dose and another three patients are treated in the next lower dosage cohort.

The recommended phase IB dose for this combined treatment will be defined as either (1) the highest dosage cohort in which six patients had been treated and there were less than two DLTs or (2) afatinib at the previously defined maximum tolerated dose as a single agent (i.e., 40 mg/d) plus dasatinib at the highest tolerated dose investigated in this indication (cohort 3). In Phase IB, a mutationally selected 20 participants (total) will be treated at the recommended dose to confirm tolerability and evaluate for early response signal.

6.3.2 Definition of Dose-Limiting Toxicity (DLT)

Toxicity is graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTCAE) version 4. The MAD is defined as the highest dose at which ≤ one (17%) of six patients experiences a DLT during the first 28-day treatment period. Only DLTs collected during the first cycle of treatment will be used for dose-escalation decision but will continue to be collected through cycle 2 and beyond. A DLT is defined as grade 4 rash, severe grade 3 rash refractory to optimal supportive care, grade 3/4 nausea/vomiting refractory to antiemetics, grade 3 diarrhea refractory to anti-diarrhea medications (see Management of Adverse Events – Diarrhea), grade 3 fluid retention refractory to diuretics and prednisone (see Management of Adverse Events – Fluid Imbalance). In addition, other grade 3/4 non-hematologic toxicity, grade 4 hematologic toxicity, or death will be considered DLTs if the event attribution is considered definite or probable by the primary investigator. Diarrhea related to a known infectious agent is not considered a DLT.

The publication demonstrated that the presence of a papulopustular skin rash strongly correlated with overall survival in patients receiving erlotinib after failing chemotherapy [150]. Patients with grade 2+ skin rash has median survival of 11.1 months compared to 7.1 months and 3.3 months for patients with grade 1 and grade 0 skin rashes, respectively. Thus, presence of a routine grade 3 rash predicts better outcome; therefore, we want to continue these patients on EGFR TKI therapy with maximal medical support for skin rash. This includes use of topical and systemic antibiotics and/or corticosteroids as described in Management of Adverse Events: Skin Rash.

6.4 Concomitant Therapy

6.4.1 Prohibited and Restricted Therapies During Study

6.4.1.1 Prohibited Therapies

Subjects requiring any of the following prohibited therapies should not be enrolled.

Bisphosphonates

Intravenous bisphosphonates will be withheld for the first 8 weeks of dasatinib treatment due to the risk of hypocalcemia. After the need for Ca^{2+} supplementation has been assessed and levels documented to be >LLN, subjects on prior bisphosphonate may be restarted with caution at the investigator's discretion.

CYP3A4

Dasatinib is primarily metabolized by the CYP3A4 enzyme. Therefore, potent inhibitors of CYP3A4 are prohibited during study; for such medications, a wash-out period of ≥7 days is required prior to starting dasatinib. Of note, afatinib does not inhibit CYP3A4 or any other cytochrome P450 enzymes. Subjects should be advised not to consume substantial quantities of grapefruit juice.

P-Glycoprotein

The adsorption of afatinib is increased by concomitant considering the dose dependence of adverse events; for safety reasons, patients requiring comedication with potent P-gp inhibitors (including amiodarone, cyclosporine, erythromycin, ketoconazole, itraconazole, quinidine, ritonavir, verapamil, tacrolimus, dronedarone, felodipine, nelfinavir, lopinavir, saquinavir, ticagrelor, diltiazem, conivaptan, clarithromycin, azithromycin, ranolazine, captopril) or inducers (including St John's wort, rifampicin, phenobarbital, tipranavir, carbamazepine) will not be entered into ongoing and planned afatinib trials. In patients requiring any of these medications after start of afatinib treatment, the decision for continuation of either drug will be based on the individual circumstances of the patient upon discussion with the responsible BI clinical monitor.

Dasatinib was shown to be a substrate of human P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in Madin-Darby canine kidney (MDCK) II cells. However, dasatinib exhibits high intrinsic permeability, and thus a meaningful impact of P-gp on its intestinal absorption upon oral administration is unlikely. Dasatinib was not an inhibitor of P-gp in Caco-2 cells and is not expected to alter the absorption and distribution characteristics of compounds that are P-gp substrates. (Paragraph reproduced verbatim from BMS Investigator Brochure v13.0 930003494, pg 25)

Drugs that may increase dasatinib plasma concentrations

CYP3A4 Inhibitors: Dasatinib is a CYP3A4 substrate. Concomitant use of dasatinib and drugs that inhibit CYP3A4 (e.g., ketoconazole, itraconazole, erythromycin, clarithromycin, ritonavir, atazanavir, indinavir, nefazodone, nelfinavir, saquinavir, telithromycin) may increase exposure to dasatinib and should be avoided.

In patients receiving treatment with dasatinib, close monitoring for toxicity and a dasatinib dose reduction should be considered if systemic administration of a potent CYP3A4 inhibitor cannot be avoided. Of note, afatinib does not inhibit CYP3A4 (refer to afatinib Investigator Brochure.).

Medications that prolong QT interval

Subjects enrolled in this study should not take or begin to take concomitant medications known to prolong the QT interval. For such medications, a wash-out period of ≥7days is required prior to starting dasatinib. (Agents which may possibly prolong the QT interval are restricted). Medications known to prolong the QT interval (Class I; see http://www.qtdrugs.org/medical-pros/drug-lists/drug-lists.htm) are: Drugs that are generally accepted to have a risk of causing Torsades de Pointes include:

- quinidine, procainamide, disopyramide
- amiodarone, sotalol, ibutilide, dofetilide
- erythromycin, clarithromycin
- chlorpromazine, haloperidol, mesoridazine, thioridazine, pimozide
- cisapride, bepridil, droperidol, methadone, arsenic, chloroquine, domperidone, halofantrine, levomethadyl, pentamidine, sparfloxacin, lidoflazine, ranozaline

Should the Investigator believe that beginning therapy with a potentially QT prolonging medication (other than the ones explicitly prohibited) is vital to an individual subject's care, the Investigator must check that the subject's prior on-therapy EKG has not shown a QTcF ≥ 480 msec in women or >470 msec in men (per ACC/AHA 2010 guidelines for prevention of torsades [151] [152]), or an increase in QTc ≥ 60 msec over the baseline value.

6.4.1.2 Restricted Therapies

Afatinib is a substrate of P-gp, and its plasma concentrations can be affected by the use of P-gp inhibitors and it is also likely that P-gp inducers could also influence afatinib plasma concentrations. The use of potent P-gp inhibitors (including Cyclosporin, Erythromycin, Ketoconazole, Itraconazole, Quinidine, Phenobarbital salt with Quinidine, Ritonavir, Valspodar, Verapamil) and potent P-gp inducers (including St John's wort, rifampicin) has to be avoided during treatment with afatinib.

Drugs that may decrease dasatinib plasma concentrations

CYP3A4 Inducers: Drugs that induce CYP3A4 activity may decrease dasatinib plasma concentrations. In patients in whom CYP3A4 inducers (e.g., phenytoin, carbamazepine, rifampicin, phenobarbital, or high-dose dexamethasone) are indicated, alternative agents with less enzyme induction potential should be used.

Drugs that may have their plasma concentration altered by dasatinib

CYP3A4 Substrates: CYP3A4 substrates known to have a narrow therapeutic index such as terfenadine, cisapride, cyclosporine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus, or

ergot alkaloids (ergotamine, dihydroergotamine) should be administered with caution in patients receiving dasatinib.

Less-potent inhibitors, inducers, and substrates of CYP3A4 are restricted.

St. John's wort (Hypericum perforatum): May decrease dasatinib plasma concentrations unpredictably. Patients may not take St. John's wort.

Antacids: Nonclinical data demonstrated that the solubility of dasatinib is pH dependent. Simultaneous administration of dasatinib with antacids should be avoided. If antacid therapy is needed, the antacid dose should be administered at least 2 hours before or 2 hours after the dose of dasatinib.

H_2 Blockers/Proton Pump Inhibitors: Long-term suppression of gastric acid secretion by H_2 blockers or proton pump inhibitors (e.g., famotidine and omeprazole) is likely to reduce dasatinib exposure. The concomitant use of H_2 blockers or proton pump inhibitors with dasatinib is not recommended. The use of antacids should be considered in place of H_2 blockers or proton pump inhibitors in patients receiving dasatinib therapy.

Medications that inhibit Platelet Function and Anticoagulants

Caution should be exercised if patients are required to take medications that inhibit platelet function or anticoagulants.

Medications that directly and durably inhibit platelet function include:

- aspirin or aspirin-containing combinations, clopidogrel, dipyridamole
- tirofiban, dipyridamole, epoprostenol, eptifibatide, cilostazol, abciximab, ticlopidine, cilostazol

Medications that directly and durably inhibit anticoagulation include:

- warfarin, heparin/low molecular weight heparin [e.g., danaparoid, dalteparin, tinzaparin, enoxaparin]
- Exceptions: low-dose warfarin for prophylaxis to prevent catheter thrombosis, and heparin for flushes of IV lines.

6.4.2 Potential for Afatinib / Dasatinib Interactions

The available literature does not provide any theoretical reason to expect that afatinib will significantly affect the metabolism or elimination of dasatinib. Dasatinib is metabolized by

CYP3A4 (primarily), flavin-containing mono-oxygenase-3 (FOM-3), and uridine diphosphate-glucuronosyltransferase (UGT). Afatinib does not inhibit either of these pathways.

Afatinib is primarily excreted unchanged as parent compound in the feces, although a minor fraction is metabolized by P-gp mechanisms. It is not a significant substrate of P450 enzymes such as CYP 3A4, nor is it metabolized by FOM-3. Therefore, it is unlikely that dasatinib will affect the pharmacokinetic profile of afatinib.

Dasatinib and P-glycoprotein

Dasatinib has been demonstrated to be a substrate of human P-glycoprotein (P-gp) in Madin-Darby canine kidney (MDCK) II cells. [153] However, dasatinib exhibits high intrinsic permeability, and thus a meaningful impact of P-gp on its intestinal absorption upon oral administration is unlikely. (BMS-354825 Investigator Brochure v13.0 11-3-10, pg 25). Dasatinib was not an inhibitor of P-gp in Caco-2 cells and is not expected to alter the absorption and distribution characteristics of compounds that are P-gp substrates.

6.5 Treatment of Persons of Childbearing Potential

Sexually active women of childbearing potential must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized.

Prior to study enrollment, women of childbearing potential (WOCBP) must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. In addition, men enrolled on this study should understand the risks to any sexual partner of childbearing potential and should practice an effective method of birth control.

All WOCBP MUST have a negative pregnancy test prior to first receiving investigational product. (see Study Calendar). If the pregnancy test is positive, the patient must not receive investigational product and must not be enrolled in the study.

In addition, all WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation. The Investigator must immediately notify Boehringer-Ingelheim and Bristol-Myers-Squibb in the event of a confirmed pregnancy in a patient participating in the study.

Female patients who are not of childbearing potential due to being postmenopausal (2 years without menses) or surgical sterilization (oophorectomy, hysterectomy, and/or tubal ligation) do not need to use contraception.

All other female patients are considered to have childbearing potential and should use adequate contraception throughout the study (from screening until end of study participation or 28 days after last dose of trial medication, whichever is later).

Acceptable methods of contraception for WOCBP include hormonal contraception and double barrier method. Double barrier method of contraception is defined as two barrier methods used simultaneously each time the patient has intercourse. Accepted barrier methods include diaphragm, female condom, cervical cap, male condom, and IUD (the diaphragm and cervical cap must be used in conjunction with spermicidal jelly/cream). If hormonal contraceptives are used, at least one barrier method should also be used. Partner vasectomy, natural “rhythm,” and spermicidal jelly/cream are not acceptable as methods of contraception.

Male patients should use adequate contraception throughout the study (e.g. condom and spermicidal jelly).

6.6 Patient Withdrawal Criteria

Protocol therapy will be discontinued at any time if any of the following situations occur: progressive disease; development of toxicity that, in the Investigator’s judgment, precludes further therapy:

1) patient refusal
2) patient lost to follow-up/noncompliance
3) intercurrent illness
4) at the discretion of the Investigator or sponsor
5) study termination

When a patient is removed from the study, the Investigator will clearly document the reason in the medical record and complete the appropriate case report form page describing the reason for discontinuation. If the reason for withdrawal from the trial is the death of the subject, the two options for categorizing withdrawal are either progressive disease or an adverse event (AE); more than one AE may be documented as a reason for withdrawal. Only one event will be captured as the cause of death. Note that death is an outcome and not an AE. Deaths unequivocally due to progression are not SAEs. All trial treatment–related toxicities and SAEs must be followed up until resolution. All subjects who have new or worsening CTC grade 3 or 4 laboratory values at the time of withdrawal must have further tests performed and the results recorded appropriately until laboratory values have returned to CTC grade 1 or 2, unless these

values are not likely to improve because of the underlying disease. In these cases, the investigators must record their opinions in the subject's medical records. Laboratory abnormalities should not be reported as adverse events unless any criterion for an SAE is fulfilled and/or the laboratory abnormality causes the subject to discontinue the study, and/or the investigator insists the abnormality should be reported as an AE.

At withdrawal, all on-going study-related toxicities and SAEs must be followed until resolution, unless, in the investigator's opinion, the condition is unlikely to resolve due to the subject's underlying disease. After withdrawal from treatment, subjects must be followed up for all existing and new drug-related AEs for 21 calendar days after the last dose of trial drug. All new drug-related AEs occurring during that period must be recorded and causality assigned, and all study-related toxicities and SAEs must be followed up for resolution where possible. Physical examination, tumor measurements, CBC with differential and CMP, and evaluation are to be performed when the patient goes off-study (EOT).

6.7 Noncompliance

All instances of noncompliance and all resulting protocol deviations will be recorded on the case report forms. Participants will be given one opportunity to fail to adhere to study requirement for return of a pill bottle and/or a cycle diary. If a second violation occurs, participants must return their previous pill bottle and /or diary before any additional study pills will be dispensed.

6.8 Accountability Procedures

Patients must bring back empty bottles and all remaining afatinib and dasatinib tablets, so these can be counted. The patients will also be required to keep a pill diary. The number of tablets taken will be recorded on the case report forms.

6.9 Study Period

Patients will be required to remain on study for the first two cycles unless they develop intolerable toxicities, have rapid progression, or fulfill the patient withdrawal criteria above. Patients with documented response or stable disease can continue therapy until progression or intolerable toxicity. A pattern of participants failing to adhere to study requirements after one or more previous violations or warnings may also result in participants discontinuing treatment (See Patient Withdrawal Criteria).

6.10 Trial Discontinuation

For reasonable cause, the Investigator or sponsors may terminate this study prematurely. Conditions that may warrant termination include, but are not limited to, the discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study or if the accrual goals are met. A written notification of termination will be issued.

6.10.1 Special Situations

Patients will discontinue treatment if they experience deterioration in left ventricular cardiac function (LVEF) to CTCAE Grade ≥3.

Patients will discontinue treatment if they are diagnosed with an ILD.

In the event of a prolonged (≥7 consecutive days) grade 2 drug-related event not listed in the table above, which is poorly tolerated by the patient, the investigator may choose to pause the medication for up to 14 days to allow the patient to recover to grade 1 or baseline followed by a dose reduction according to a 5 days on, 2 days off schedule.

Pregnancy: See Treatment of Women of Child-Bearing Potentials for policy regarding mandatory contraception. In rare cases, pregnancy might occur in clinical trials. Once a female subject has been enrolled into the clinical trial, after having taken study medication, the investigator must capture any drug exposure during pregnancy. The investigator will follow the pregnancy until outcome is known. An SAE report must be forwarded to the pharmaceutical company if the outcome of the pregnancy results in an abortion/miscarriage or the occurrence of any other SAE(s).

Patients will discontinue treatment if they are diagnosed with pregnancy.

6.11 Management of Adverse Events

6.11.1 Rash

A proactive and early approach to management of rash is crucial. Rash can be managed by a variety of treatment options to relieve symptoms and reduce the rash. A guideline table for management of papulopustular skin rash is provided in the Appendix: Management of Skin Rash.

The recommendations for management are as follows:

General/Prevention: strict sun protection; use of a sunscreen of Sun Protection Factor 15 (SPF 30 or higher, preferably containing zinc oxide; use of a thick, alcohol-free emollient cream; avoid harsh detergents, avoid using a solarium.

It is optional and recommended that participants receive a prescription for minocycline 50 mg or 100 mg bid for the initial 8 weeks of afatinib treatment to prevent papulopustular skin rash, per dermatology guidelines. [154]

CTCAE Grade 1 rash: Initiate topical hydrocortisone (1% or 2.5%) cream and clindamycin 1% gel should be used.

CTCAE Grade 2 rash: Refer to Appendix, Management of Skin Rash, Relief from major symptoms caused by CTCAE Grade 2 skin related adverse events should be achieved by a combination of local and systemic therapies. In Phase II trials, this approach enabled continued dose for the majority of patients requiring dose reduction for skin toxicity. Specifically we recommend including:

1) Systemic antibiotics (minocycline 100 mg PO bid or doxycycline.).

2) Topical treatment (hydrocortisone 2.5% cream, clindamycin 1% gel, pimecrolimus 1% cream).

Optional:

1) Oral or topical antihistamines (diphenhydramine, hydroxyzine, chlorpheneramine, etc.).

2) Oral prednisone (short term i.e., <14 days treatment) may be added at investigator's discretion.

Any of the following additional therapies can be prescribed to help with rash: minocycline, bactrim, topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisone (short course) at discretion of investigator. Systemic and topical treatment should be initiated at the start of CTCAE Grade 2 rash and continue until improvement or resolution to CTCAE Grade ≤1. If grade 2 rash persists for ≥ 7 days despite treatment and is poorly tolerated by the patient, the investigator may choose to pause treatment for up to 14 days followed by a reduction in the dose of afatinib.

CTCAE Grade 3 rash: Treatment algorithm should follow CTCAE Grade 2 rash algorithm as outlined above, with the exception of a Severe Rash, defined as outlined below:

Severe Grade 3 rash: In the event of severe, generalized erythroderma or macular, papula or vesicular eruption; desquamation covering more than 50% of body surface area (BSA), treatment with afatinib and dasatinib should be paused until recovery to CTCAE Grade ≤2. This is in accordance with Lacouture *et al*, *Expert Rev. Anticancer Ther* 13(6) 2013. Treatment should be resumed at a reduced dose per Appendix, "Papulopustular Skin Rash". If CTCAE Grade ≥3 rash does not resolve to CTCAE Grade ≤2 after 14 days of stopping afatinib treatment and despite optimal supportive care, the patient should not receive any further treatment with afatinib and the End of Treatment visit should be scheduled.

CTCAE Grade 4: Patients experiencing Grade 4 skin toxicity should receive expedient medical evaluation and cessation of all study drugs.. If Phase IA during DLT evaluation window, a DLT will be registered. Otherwise, Grade 4 rash that has not improved to ≤2 by 14 days, should be discontinued from study and EOT performed.

6.11.2 Diarrhea

Oral hydration is essential regardless of severity; appropriate rehydration (1.5 L/m^2/day plus equivalent of actual fluid loss) and electrolyte replacement has to be ensured in the event of CTCAE Grade 2 and Grade 3 adverse events. See "Appendix, Management of Diarrhea". Participants should be counseled to purchase or receive prescriptions for Imodium because of the likelihood of experiencing any episode of diarrhea. This counseling should take place at C1D1, the initiation of study drug.

For Grade 1, if any diarrhea is experienced (CTCAE Grade 1), two 2 mg loperamide tablets should be taken immediately, followed by one 2 mg tablet with every loose bowel movement, up to a maximum daily dose of 10 tablets (20 mg). Both dasatinib and afatinib should continue at the current doses. In the event of diarrhea patients should be advised to avoid lactose-containing products or any foods known to aggravate diarrhea. In particular, spicy foods should be avoided and meals divided to 6 small meals per day.

For Grade 2, patients should begin loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until diarrhea free for 12 hours). Lomotil (2.5 mg tablets, 1-2 PO q6hr) should be added at the discretion of the treating physician, and is strongly recommended if patients have previously been treated with loperamide. Both dasatinib and afatinib should continue at the current doses. Intravenous fluids may be considered in select cases.

For Grade 3, or CTCAE Grade 2 diarrhea lasting ≥ 3 days (72 hours) despite adequate antidiarrheal treatment, in Phase IB afatinib and dasatinib must be paused until recovery to CTCAE ≤ Grade 1. Maximal medical support with Imodium and Lomotil should be followed as

in Grade 2. Of note, if Grade 3 diarrhea persists after 2 days of optimal supportive care and cessation of study drug, DLT should be registered (See DLT definition) and will be recorded, and EOT performed with appropriate interval.

After resolution of Grade 3 diarrhea to ≤1, both dasatinib and afatinib can be reintroduced at the reduced dose according to the dose reduction scheme of 5 days on, 2 days off, along with prophylactic loperamide and/or lomotil. If grade 3 diarrhea again recurs, the patient again temporarily discontinued from both agents, and for Phase IA a DLT must be registered. For Phase IB, when diarrhea improves to Grade ≤1, the patient may again re-attempt dasatinib & afatinib. If despite optimal supportive care and a treatment pause, diarrhea does not resolve to CTC Grade ≤1 within 14 days, the patient must not receive any further afatinib or dasatinib treatment and EOT should be performed at appropriate interval.

For Grade 4 diarrhea, a DLT should be registered and patients should be discontinued from study treatment. No reattempt of treatment at the next lowest dose cohort is permitted.

6.11.3 Nausea and Vomiting

Nausea and vomiting may significantly affect patients' adherence to the treatment and their quality of life. In order to reduce the occurrence and the intensity of emesis, the patients should be treated with an aggressive antiemetic program, as recommended in Appendix: Management of Nausea and Vomiting.

In case of nausea and/or vomiting ≥ CTCAE grade 2, appropriate hydration (1.5 L/m^2/day plus hydration deficit) must be ensured.

6.11.4 Pulmonary

Unexplained dyspnea, either new or progressive, should be aggressively evaluated. Careful assessment of all patients with an acute onset and/or unexplained worsening of pulmonary symptoms (dyspnea, cough, fever) should be performed to exclude ILD. Both afatinib and dasatinib should be interrupted pending investigation of these symptoms. If interstitial lung disease is diagnosed, study drug should be permanently discontinued and appropriate treatment instituted, including verbal discussion with, and referral to, a pulmonologist.

If doses have to be held, those doses will be skipped and will remain on schedule. Any doses that are inadvertently skipped will not be replaced and the patient will remain on schedule.

6.11.5 Fluid Imbalance

Dasatinib has been reported to cause transudative pleural effusions in patients with cancer. For new or worsening pleural effusions in patients, diuretics and/or a brief course of corticosteroids should be instituted according the recommended guidelines (See, "Appendix: Management of Localized Edema". For effusion that continue or worsen despite use of diuretics/corticosteroids, thoracentesis should be performed to confirm the nature of the effusion including LDH and protein levels and cytology should be evaluated to confirm that the effusion is not malignant. For symptomatic effusions that recur after thoracentesis, patients should be considered for either (i) pleurodesis with talc or betadine or (ii) indwelling chest tube. This decision will be left to the discretion of the treating physician. Patients who develop grade 3 pleural effusions (symptomatic and supplemental oxygen, >2 therapeutic thoracentesis, tube drainage, or pleurodesis) should have dasatinib held for up to 14 days and then may restart dasatinib at the next lower dose. Patients who then redevelop grade 3 pleural effusions should be discontinued from dasatinib and afatinib and DLT registered.

6.11.6 General Dose Modifications Beyond Cycle 2

Given the chronic nature of these medications and the possibility of developing DLT or intolerable toxicities beyond cycle 1, allowances are made for dose modifications beyond those discussed above for afatinib and/or dasatinib. If a patient develops a DLT or intolerable grade 1 or 2 non-hematologic toxicity after cycle 1, dose modifications of afatinib and/or dasatinib are allowable at the treating physician's discretion. Treatment with afatinib and/or dasatinib can be withheld until toxicity resolves to less than or equal to grade 2 (for patients with documented DLT) or when the patient's intolerable toxicity resolves. The dose of the withheld compound(s) can then be modified at the treating physician's discretion. See Appendix: Toxicity Tables for general recommendations. For dose interruption of afatinib, re-treatment can either be at the same dose or reduced one level, viz. from 40 mg daily to 30 mg daily. For dose interruption of dasatinib, re-treatment can be at the same dose or reduced one level, viz. from 100 mg daily to 80 mg daily. Patients who fail to recover to grade 0/1 or tolerable grade 2 from a treatment related AE within 14 days or those who require a third dose reduction should discontinue therapy.

7 OBSERVATIONS

7.1 Methodology

7.1.1 Plasma Collection and Processing

Blood samples are to be collected from an indwelling catheter or by direct venipuncture. If a catheter is used for blood collection, then approximately 10 mL of blood should be withdrawn initially and discarded.

7.2 Correlative Science

7.2.1 Stored Plasma Samples

Previous studies have indicated that SRC inhibitors can reduce tumor production of p-SRC, VEGF and IL-8 that are important factors for tumor angiogenesis. Blood will be collected at the time of screening or day 1, and again on day 29 to assess for future correlative markers with a purple-top EDTA tube. These samples will be processed and analyzed for plasma-based molecular markers. Serum/Plasma will be centrifuged for 1 minute at 12,000 rpm to pellet any floating debris/cells, and then transferred to a fresh microcentrifuge tube and immediately frozen and stored at –80°C.

These samples will be processed and analyzed for plasma-based molecular markers. Specifically, plasma is extracted and assessed for epidermal growth factor receptor (EGFR) mutation type and copy number. This testing is done using digital droplet PCR with specifics primers for EGFR L858R, Exon 19 del, and T790M. This testing is performed by collaborator (Biodesix), using an established and executed material transfer agreement (Biodesix-Moffitt 113016). Coded plasma aliquot samples are shipped to collaborator by Moffitt Tissue Core, and coded .csv file containing the number of relevant reads per pooled aliquot is then sent back to the investigator by the collaborator.

7.2.2 Radiomic Analysis for Imaging Predictive Markers

The aim is to use state of the art image acquisition and feature extraction analysis to obtain a radiomic profile that predicts the clinical activity of dual tyrosine kinase inhibitors in patients

with NSCLC. All Radiomics analysis will be performed retrospectively. Trial participants will not be subject to any additional direct testing, imaging, or charges as part of this component of the correlative science. Only imaging data available via Moffitt PACS system will be analyzed as part of this correlative endpoint. Participants are encouraged to have baseline and surveillance imaging performed at the Cancer Center.

7.2.2.1 Assessment

Tumor radiomics involves the high throughput segmentation of tumor volumes from anatomic images, the extraction of quantitative features from rendered volumes which are used to write a report. Segmentation is provided by a research scientist (Dr. V Kumar) in consultation with a radiologist (Dr. E. Outwater and/or Dr. D. Klippenstein) and a computer expert (Dr. Yuhua Gu). Segmentation is achieved through a one-click region growing routine. Because this often fails to segment the tumor on first pass, multiple operator inputs are required. To minimize the operator inputs and optimize the segmentation we developed an algorithm that performs the region growing, followed by region shrinking. In this shrunken volume, 20 different seed points are inserted on a Cartesian grid and these are used simultaneously as seed points. This has been repeated 20 different times each for 19 different patients. These results have been compared to the same lesions independently segmented by 5 different radiation oncologists, with a linear correlation coefficient of 0.926. Work is ongoing to improve the multi-seed point segmentation. For the current study, segmented volumes are overread by thoracic radiologists. Over 300 features are extracted in the report.

Reconstructed data will be used to determine RECIST 1.1 response if available. Coded and reconstructed data will be sent to Moffitt for central review with annotated markup of lesions used in RECIST v1.1 measures. If possible, we will endeavor to obtain raw (unreconstructed) data for archival storage, although the logistics have yet to be worked out and thus, is not a component of the protocol.

For the purpose of the Radiomic analysis, only the diagnostic CE-CTs obtained prior to therapy and at the time of disease progression will be utilized. Additional CE-CTs will be obtained as described in the study calendar for the determination of the primary and secondary protocol objectives.

7.2.2.2 Data Recording

DICOM data will be transferred to MCC via CDs or secure FTP, and the research team headed by Eric K. Outwater, MD will proceed to segment. He will overread these segmented volumes to ensure compliance with standard practice. From these segmented lesions,

volumes will be rendered and reports generated on all 233 quantitative features. A second level of Quality Assurance will involve comparison of the RECIST v1.1 metrics generated by automated feature extraction to the values provided by individual radiologists at the initiating site. At the MCC these data will be parsed to answer the following questions:

1. Is the range of values for SCLC features similar or different to that observed for NSCLC?
2. Can pre-therapy features be used to predict response to TKIs?
3. Can changes in features following therapy be used to predict outcome to TKIs?
4. Do any CT features correlate with molecular biomarkers being collected in this study?
5. Do biomarkers add to the diagnostic, or predictive accuracy of the image features?
6. Are any features more reproducibly changed compared to RECIST?

Data generated from these methods will be entered into a nascent CaBIG complaint database that will be made accessible by the trial statistician.

7.3 Assessment of Safety

7.3.1 Safety Evaluation Overview

Patients are seen at screening, C1D8, C1D15 for the first cycle in Phase IA for evaluating of toxicity. This includes a complete history and physical exam including weight, vital signs, assessment of performance status and laboratory evaluation including complete CBC with differential, platelet count, and CMP.

For purposes of dose escalation, only DLTs collected during the first 28 day cycle will be collected and analyzed. However, during cycle 2 and for treatment beyond cycle 2, DLTs will continue to be collected during the visits to the treating physician. (see Table 8: Dose Escalation). While there is no formal process for analyzing these data, these data will be periodically reviewed by the study PI to assess the potential of delayed toxicities of therapy. See Section: DLT definition, for definition of DLT. In cases in which a Grade 3 toxicity is awaiting resolution with supportive care for DLT determination, the dose escalation decision will be postponed until the evaluation time for DLT determination has elapsed.

Toxicity is graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4

(http://ctep.cancer.gov/forms/CTCAEv4.pdf).

7.3.2 Adverse Events

During the screening phase of the trial, the patient's condition will be assessed (e.g., documentation of history / concomitant diagnoses and diseases), and subsequently all relevant changes from baseline will be noted.

The definition of adverse events (AEs) and serious adverse events (SAEs) can be found in section, "9.2 Definition of Adverse Events." The most common adverse events are expected to be gastrointestinal (diarrhea, nausea, vomiting), papulopustular skin rash, and fluid retention. The management of these toxicities is outlined in Appendix: Toxicity Tables.

Patients will be required to report spontaneously any adverse events (AEs) as well as the dates of onset and end of these events. Specific questions will be asked wherever required or useful to more precisely describe an AE and to allow a grading according to CTCAE, Version 4. A carefully written record of all AEs shall be kept by the investigator in charge of the trial.

Records of AEs shall include data on the date of onset, end date, and CTCAE grading of the event as well as any treatment or action required for the event and its outcome. Regular and continuing assessment of safety will be performed at least once per course during the first six courses and every three weeks thereafter. Dose reduction schemes are provided in specified tables (See Management of Adverse Events) for patients who experience specified adverse events and who, at the discretion of the investigator, could derive benefit from continuing treatment on the protocol.

- Adverse events which are not yet recovered at the End of Treatment visit will be followed up until recovery, or in case of persistence sufficient characterization of the toxic effects has been achieved, then Bristol-Myers-Squibb and Boehringer Ingelheim GmbH may agree not to pursue them further.

- Adverse events that occur between cessation of treatment and End of Treatment visit will only be reported if they are considered related to trial medication or procedures by the Investigator. Total observational period for AE reporting is 30 days after discontinuation of study drug.

- Adverse events occurring after the End of Treatment visit will be reported only if considered serious (SAEs) and related to trial medication or procedures.

Data regarding deaths which are not related to trial medication will be collected for the purposes of assessing the overall survival endpoint but these deaths will not be reported as SAEs.

7.3.3 Time Period Reporting Requirements

- Beginning of Treatment to End of Treatment visit: Report all AEs and SAEs regardless of relatedness. This includes all deaths.
- End of Treatment visit to final observation: Report AEs and SAEs which are considered related to study treatment or procedures.

Death should not be reported as an SAE unless considered related to study treatment or procedures (because death is an endpoint and will be followed-up separately). See Section: Section: Adverse Event Reporting Period for comprehensive review of reporting procedures.

Definitions and requirements for documentation and reporting of AEs and serious adverse events (SAEs) during a trial in OnCore are provided in "Definition of Adverse Events."

7.3.3.1 Hospitalization

Patients may be hospitalized for administrative reasons during the trial. These and other hospitalizations planned at the beginning of the trial need not be reported as SAEs if they have been documented at the screening visit and have been performed as planned.

Changes observed in safety tests including blood pressure, pulse rate, electrocardiogram (EKG) and laboratory tests will be recorded as AEs and graded according to CTCAE, if they are not associated with an already reported AE, symptom or diagnosis, and meet at least one of the following criteria:

- Action is required and taken with the investigational drug, i.e., dose reduction or treatment discontinuation.
- Treatment is required (i.e., a concomitant medication is added or changed).

7.3.3.2 Worsening of pre-existing conditions

Expected fluctuations or expected deterioration of the underlying disease will not be recorded as an AE. If progressive disease occurs and is associated with symptoms or meets one of the seriousness criteria, the signs and symptoms of progressive disease will be reported as an adverse event or a serious AE (if applicable).

The only exception to the above is in the event of a death which is attributed to progressive disease but where the signs and symptoms are not available. In this situation it is acceptable to report the progressive disease as the serious AE.

A pre-existing condition present at baseline, which remains unchanged during the trial, does not need to be recorded as adverse event. However, any worsening of any pre-existing baseline condition should be reported as an adverse event. Examples of worsening of a preexisting condition that should be recorded as an AE are given below:

- Worsening of condition meets the criteria for an SAE
- Action is taken with the investigational drug (i.e. dose is reduced or treatment is discontinued)
- Treatment is required (concomitant medication is added or changed)
- The investigator believes a patient has shown a clear deterioration from baseline symptoms

7.3.3.3 Assessment of healthcare resource use

If patients are hospitalized due to adverse events, the reason for hospitalization, the duration of hospital stay, emergency room admission and time in the intensive care unit will be documented in OnCore®. Adverse event-related outpatient visits and interventions will also be documented in OnCore®. Information on caregiver support (home care), hospital, and outpatient visits (other than scheduled visits) collected in the OnCore® will inform on healthcare resource use required to treat the trial indication and adverse events observed during the trial.

7.3.3.4 Laboratory investigations

Blood samples will be collected at the time points specified in the Study Calendar and analyzed in a laboratory facility at (or close to) the investigational site. Safety laboratory examinations include hematology and biochemistry. In case of neutropenia, blood will be examined as clinically indicated at the discretion of the investigator until recovery.

For patients receiving study drug, the decision to continue treatment will be based on assessment of laboratory parameters. Therefore the results of these assessments should be available and assessed on the day of treatment prior to commencing treatment.

Safety laboratory assessment will include at least the following parameters:

- **Hematology:** Red blood cell count (RBC), neutrophils, hemoglobin, hematocrit, white blood cell count (WBC) and differential, platelets.

- **Biochemistry:** Glucose, sodium, potassium, calcium, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase, total bilirubin, urea, magnesium.

- **Pregnancy test** At screening, β-HCG testing in urine or serum will be performed in women of childbearing potential.

7.3.3.5 Physical examination and performance score

A physical examination will be performed at screening and at the time points specified in the Study Calendar.

A full physical exam serves as a clinical tumor assessment and should include a cardiopulmonary examination, examination of the regional lymph nodes, examination of the abdomen, and an assessment of the mental and neurological status. Additional symptoms that have not been reported during a previous examination should be clarified. Wherever possible, the same investigator should perform this examination.

Measurement of height (in cm) and body weight (in kg) and the evaluation of the ECOG performance score will be performed at the time points specified in the Study Calendar.

7.3.3.6 Vital signs

Vital signs (blood pressure, pulse, and respiratory rate after 2 minutes supine rest) and temperature will be recorded at the screening visit and at the time points specified with the physical examination.

7.3.3.7 EKG

A 12-lead resting EKG will be performed at the time points specified in the Study Calendar. EKGs will be performed using a digital EKG machine.

The investigator should review the EKG data at the time of the visit and this will be used to make decisions on eligibility for the study and treatment.

7.3.3.8 Left ventricular function assessment

Left Ventricular Ejection Fraction (LVEF) as measured by echocardiography or MUGA scan will be assessed at time points specified in the Study Calendar. The same method of measurement should be used throughout the study.

- **MUGA Scan -** The Multiple Gated Acquisition scan (MUGA) is recommended as a non-invasive method for the assessment of diseases of the heart muscle. It is used for the monitoring of the ejection fraction of the cardiac ventricles, especially the left ventricular ejection fraction (LVEF).

- **Echocardiography (ECHO)** Echocardiography will be performed to assess the LVEF according to the standard guidelines of the American Society of Echocardiography (ASE). http://asecho.org/Guidelines.php

7.3.4 Data and Safety Monitoring Committee (DMSC)

A Data and Safety Monitoring Committee (DSMC) will be set up for this study in order to ensure its ongoing safety. Safety review meetings will be held according to the DMC charter: approximately every 3 months (depending on the rate of enrolment) and at the time of data review.

Inclusion of patients in the study will continue during the scheduled meetings of the DMC. Decisions on trial termination, amendment or cessation of patient recruitment, based on safety or outcome findings will be made after recommendations from the DMC have been assessed by the sponsor. (For comprehensive description of DSMC procedures, see Data Safety Monitoring Board).

7.4 Assessment of Efficacy

7.4.1 Evaluation of Response

Tumor size is assessed via computed tomography at day #50 ± 7 days, and every 56 days ± 7 days thereafter. Magnetic resonance imaging may be used only in those select cases in which such imaging is preferable for specific metastases, such as the brain or pelvis. At investigator discretion, Whole Body PET/CT scan may be substituted for CT scan for evaluation of responses.

The Phase IA group will include participants with any evaluable disease, including small lesions (longest diameter <10 mm), or lytic bone metastases, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory skin metastasis, lymphangitis pulmonis, and cystic lesions. These disease elements are not necessarily included in formal RECIST criteria. Since there may be cases in which the response type is radiographically or clinically inconclusive, it is the responsibility of the clinician (with shared input from principle investigator) to decide upon continuation of study drug.

For the Phase IB extension cohort only, Modified Response Evaluation Criteria in Solid Tumors (RECIST v 1.1) are used to determine tumor response and disease progression. (See Response criteria).

7.4.2 Response Criteria for Phase IA

7.4.2.1 Baseline criteria

Measuring of tumor lesions at baseline. The Phase IA portion of the trial requires the presence of measurable lesions at baseline.

Definition of "Measurable Lesions":

- Lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 10 mm by CT scan and MRI (no less than double the slice thickness). 10 mm caliper measurement by clinical exam (when superficial).
- Lytic bone lesions, with an identifiable soft tissue component, evaluated by CT or MRI, can be considered as measurable lesions if the soft tissue component otherwise meets the definition of measurability previously described.
- "Cystic Lesions" thought to be cystic metastases can be considered as measurable lesions, if they meet the definition of measurability. However, if non-cystic lesions are present in the same patient, these should be preferably selected for assessment.
- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.
- All other lesions will be judged as "non-measurable", including lesions <10 mm on spiral CT.

All measurements will be recorded in metric notation, using a ruler or calipers. All baseline evaluations will be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

7.4.2.2 Specifications by methods of measurements

The same method of assessment and the same technique will be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment. Spiral CT of the chest and abdomen will be used for the

initial assessment, for assessment of disease response, and, whenever possible, for the documentation of recurrent and/or progressive disease.

CT and MRI; CT (applies only for recurrent/progressive disease) might be the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to the chest and abdomen. Head & neck and extremities usually require specific protocols. These scans will be read by thoracic radiologists trained in RECIST v 1.1 criteria at the treating cancer center.

Clinical lesions (applies only for recurrent/progressive disease): Clinical lesions will only be considered measurable when they are superficial (e.g. skin nodules, palpable lymph nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended.

Chest X-ray (applies only for recurrent/progressive disease): Lesions on chest X-ray are acceptable as measurable lesions only when they are clearly defined and surrounded by aerated lung. However, CT is recommended and preferred as the tool for response measurement in this study. Chest x-ray will be used for interim surveillance for pleural effusion, a known dasatinib-associated toxicity.

Ultrasound (applies only for recurrent/progressive disease): When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions that are clinically not easily accessible. It is a possible alternative to clinical measurements for superficial palpable nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

7.4.2.3 Tumor response evaluation

To assess objective response, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Patients with any clinically or radiologically evaluable disease will be included on Phase IA of this protocol. However, only patients with measurable disease by RECIST v. 1.1 criteria will be included in the Phase IB extension. Objective tumor response in this IB group is a secondary endpoint. Measurable disease is defined by the presence of at least one measurable lesion as defined above. If the measurable disease is restricted to a solitary lesion, its neoplastic nature must be confirmed by histology for participation in this study. Evaluation should follow Table 10.

For participants with measurable disease, up to 5 lesions representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present' or 'absent.'

7.4.3 Response Criteria for Phase IB

7.4.3.1 RECIST v.1.1 criteria

Evaluation of Target lesions:

Response Criteria for Phase IB will follow RECIST v.1.1, posted at http://www.recist.com/recist-in-practice/01.html. The criteria are provided below for reference purposes only. In the advent of any inconclusive or discordant finding, the most updated RECIST v1.1 criteria will hold precedence. In the advent of any incremental development of a newer RECIST version, a protocol amendment will be required to change from RECIST version 1.1.

Complete Response (CR is defined as disappearance of all target lesions.

Partial Response (PR) is defined as at least a 30% decrease in the sum of LD of target lesions taking as reference the baseline sum LD.

Progressive Disease (PD) is defined as at least a 20% increase in the sum of LD of target lesions taking as references the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Stable Disease (SD) is defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

Evaluation of Non-Target lesions:

Complete Response (CR): Disappearance of all non-target lesions (tumor markers are not applicable).

Non-Complete Response (non-CR): Persistence of one or more non-target lesion (tumor markers are not Non-Progression (non-PD).

Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non- target lesions (although a clear progression of "non target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel or study chair).

Evaluation of Best Overall Response:

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 10: Best response

Target Lesion	Non-Target Lesion	New Lesion	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note:

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time will be reported as “symptomatic

deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

Conditions that may define "early progression, early death, and inevaluability" are study specific and should be clearly defined in each protocol (depending on treatment duration, treatment periodicity). For the purpose of this protocol, disease evaluation for response and survival will start from day 1 of therapy. Patients not receiving their first dose of therapy will be censored.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

7.4.3.1.1 **Definition of Evaluable Participants**:

Participants are considered evaluable who receive their first dose of therapy. Non-evaluable participants are those who do not receive the first dose of therapy. Participants who withdraw consent or discontinued due to protocol violations prior to the first evaluation are not considered evaluable for efficacy.

For other definitions, see Section 7.4.3.2.1 "Progression Definitions".

7.4.3.2 Confirmatory measurement / duration of response

Confirmation. Confirmation of response is not required for this study.

Duration of overall response of Progression Free Survival. The duration of overall response is measured from the time measurement criteria are met for CR/PR/SD (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease. Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started. Stable disease will be assessed for induction chemotherapy and defined as the time interval between the initial CT (weeks –4 to –1) and the first follow-up CT (week 8).

Response review. Response is the secondary endpoint in this study. We will periodically (approximately every 3 months) review all studies and charts on the patients in the study and review the measurements and data obtained. Measurements are performed at a picture/archive/communication/system (PACS) workstation using standard lung (W 2000; L –600), and soft tissue (W 350; L 20) window and level settings. This system allows for a computerized measurement of target lesions.

Patients will be censored at the date of last contact if the investigator is no longer able to contact a patient or caregiver, and vital status cannot otherwise be determined, provided that no other information indicates that the patient was near death at that point.

7.4.3.2.1 Progression Definitions

Situation Outcome	**Decision**	**Date of PFS or censoring**
No baseline tumor assessment	censored	Date of treatment
Progressed (no missed radiological assessments)	event	Date of PD
Non-PD, death before next scheduled assessment	event	Date of death
Non-PD, and one missed assessment, death or progression after date of missed assessment, but before a second scheduled assessment	event	Date of PD or death
Non-PD, more than one consecutive missed assessment, death or progression after date of second missed assessment	censored	Date of last imaging before missed assessment
New anti-cancer medication before progression or death	censored	Date of last imaging before new medication
Death before the scheduled date of first imaging event	event	Date of death
No imaging performed post-baseline, patient dies between first and second scheduled	event	Date of death

assessments		
No imaging performed post-baseline, patient dies after second scheduled assessment	censored	Day of treatment
No imaging performed post-baseline, vital status is unknown or patient is known to be alive censored	censored	Day of treatment
Alive and not progressed according to central review (no missed radiological assessments)	censored	Date of last imaging

7.4.4 Reporting of Results

Every report will contain all patients included in the study. For the response calculation the report will contain a section with all eligible patients. Another section of the report may detail the response rate for evaluable patients only. However, a response rate analysis based on a subset of patients will explain which patients were excluded and for which reasons. Confidence limits (95%) will be given whenever possible.

8 STUDY DRUG(S) SPECIFICATIONS

8.1 Product Identification

8.1.1 Dasatinib

The following investigational product, BMS-354825-03 (dasatinib), will be supplied by Bristol-Myers Squibb Pharmaceutical Research Institute in two different strengths:

BMS-354825-03 50 mg film coated tablets, biconvex, round, white to off-white in appearance with “50” debossed on one side.

BMS-354825-03 20 mg film coated tablets, biconvex, round, white to off-white in appearance with “20” debossed on one side.

8.1.2 Afatinib

The following investigational product, BIBW 2992 (afatinib), will be supplied by Boehringer-Ingelheim Pharma GmbH in three different strengths:

BIBW-2992 40 mg film-coated tablet light blue and of round, biconvex, bevel-edged with “40” debossed on one side.

BIBW-2992 30 mg film-coated tablet, biconvex, bevel-edged, oval, and dark blue with “30” debossed on one side.

BIBW-2992 20 mg film-coated tablet, biconvex, bevel-edged, oval, and light blue with “20” debossed on one side.

8.2 Packaging and Labeling

Dasatinib will be labeled in open-label fashion. Each bottle will be labeled with a two-panel label. Description of the contents, batch number, container number, storage conditions and caution statements required by country regulations will be on the label. The second panel will have batch number, container number.

Afatinib will be labeled in open-label fashion. Each bottle will be labeled with a two-panel label. Description of the contents, batch number, container number, storage conditions and caution statements required by country regulations will be on the label. The second panel will have batch number, container number.

8.3 Handling and Dispensing of Investigational Product

Investigational products should be stored in a secure area according to local regulations. It is the responsibility of the Investigator to ensure that investigational product is only dispensed to study subjects. The investigational products must be dispensed only from official study sites by authorized personnel according to local regulations.

It is recommended that investigational products should only be handled by the subject. While the risk for dermal exposure is considered minimal, it is recommended that only the study subject handle the study medication. In particular, pregnant women or women who are breastfeeding should not handle the study drug. Also children who are not study participants should not handle the drug. If caregivers must handle or come in contact with the drug, it is advised that protective gloves be worn.

Dasatinib and afatinib should be administered as an oral dose as per the protocol. Subjects do not require fasting at the time of dasatinib consumption. Afatinib should be taken one hour before, or two hours after a meal. Grapefruit juice should not be consumed during study drug therapy, as P450 enzyme inhibition may increase drug exposure.

Bristol-Myers Squibb will be responsible for assuring that the quality of BMS-354825-03 is adequate for the duration of the trial. Boehringer-Ingelheim Pharma GmbH will be responsible for assuring that the quality of BIBW-2992 is adequate for the duration of the trial.

Investigational product should be stored in a secure area, at 59°F to 77°F (15°C to 25°C).

The Investigator (or assigned designee, i.e., study pharmacist) will dispense the proper number of each strength tablet to the subject to satisfy dosing requirements for the study. The containers provided to the subject should be labeled with proper instructions for use. Subjects should be instructed to return all unused drug to the site in the same container. Re-supplies can be obtained by completing the SRC re-supply request form.. Re-supply requests need to be submitted at-least 2 weeks before the expected delivery date.

The lot numbers, dosing start dates and the number of tablets for each dosage strength must be recorded on the drug accountability pages of record for the site. The subject must be instructed to return all unused study medications in the provided packaging at each subsequent visit.

The Investigator must be satisfied the subject returned or accounted for all unused medication before additional medication is dispensed. If the number of tablets used is substantially different from the number of tablets dispensed, the subject must be counseled on how study therapy should be taken. If such deviations persist, the Investigator may consider discontinuing the subject for non-compliance.

Investigational product should be stored in a secure area according to local regulations. It is the responsibility of the Investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations.

The Investigator should ensure that the investigational product is stored in accordance with the environmental conditions (temperature, light and humidity) as determined by the Sponsor and defined in the Investigator Brochure or SmPC/reference label.

8.4 Investigational Product Records at Investigational Site(s)

It is the responsibility of the Investigator to ensure that a current record of investigational product disposition is maintained at each study site where investigational product is inventoried and disposed. Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received and placed in storage area.
- Amount currently in storage area.
- Label ID number or batch number and use date or expiry date.
- Dates and initials of person responsible for each investigational product inventory entry/movement.
- Amount dispensed to and returned by each subject, including unique subject identifiers.
- Amount transferred to another area/site for dispensing or storage.
- Non-study disposition (e.g., lost, wasted, broken).
- Amount returned to Sponsor.
- Amount destroyed at study site, if applicable.
- Retain samples sent to third party for bioavailability/bioequivalence, if applicable.

Investigational product dispensing record/inventory logs and copies of signed packing lists must be maintained at the investigational site. Batch numbers for afatinib and dasatinib must be recorded in the drug accountability records.

8.5 Return and Destruction of Investigational Product

8.5.1 Return of Investigational Product

Upon completion or termination of the study, all unused and/or partially used investigational product must be returned to BI or BMS, if not authorized by BI or BMS to be destroyed at the site.

All investigational products returned must be accompanied by the appropriate documentation and be clearly identified by protocol number and study site number on the outermost shipping container. Returned supplies should be in the original containers. Empty containers should not be returned. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept. The return of unused investigational product(s) should be arranged by the responsible Investigator at the site.

8.5.2 Destruction of Investigational Product

If investigational products are to be destroyed on site, it is the Investigator's responsibility to ensure that arrangements have been made for disposal and written authorization has been granted by BMS or BI, and that procedures for proper disposal have been established

according to applicable regulations, guidelines, and institutional procedures. Appropriate records of the disposal must be maintained. The unused investigational products can only be destroyed after appropriate instruction by BMS or Boehringer-Ingelheim.

9 ADVERSE EVENTS & DATA SAFETY MONITORING COMMITTEE (DSMC)

The safety of dasatinib and afatinib will be assessed through collection and analyses of adverse events (AEs) and laboratory tests.

9.1 Specification of Safety Variables

Safety assessments will consist of monitoring and recording protocol-defined adverse events (AEs) and serious adverse events (SAEs); measurement of protocol-specified hematology, clinical chemistry, and urinalysis variables; measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study drug(s).

Death as a result of disease progression is only to be assessed as efficacy measures and not as AEs or SAEs.

9.1.1 Definition of Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational (medicinal) product or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with NSCLC that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as biopsies)
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period

- Diagnoses and/or symptoms associated with NSCLC should be reported as AEs if they worsen or change in character. Clinical progression of NSCLC should not be reported as an AE.
- Worsening of the underlying disease or of other pre-existing conditions will be recorded as an AE in the CRF.
- Select events, regardless of grade, will be recorded as an AE: diarrhea, skin rash, thrombocytopenia, neutropenia, and AST/ALT, bilirubin or creatinine elevation,.
- Other changes in vital signs, ECG, physical examination and laboratory test results will not be recorded as an AE in the CRF
    - unless there are clinical signs or symptoms, or:
    - they required clinical intervention or treatment.

9.1.2 Protocol-Specified Significant Events

Protocol-specified significant events are to be reported in an expedited manner to BI similar to Serious Adverse Events, even if they do not meet any of the seriousness criteria. The following events are considered as afatinib protocol-specified significant events:

Hepatic injury defined by the following alterations of liver parameters (measured in the same blood draw sample):

- For patients with normal AST / ALT and total bilirubin at baseline: an elevation of AST and/or ALT above >3 fold ULN combined with an elevation of total bilirubin above >2 fold ULN.
- For patients with baseline abnormal AST/ALT : an elevation of AST and/or ALT above >5 fold ULN combined with an elevation of total bilirubin above >2 fold ULN.

Patients showing these lab abnormalities need to be followed up appropriately.

9.1.3 Serious Adverse Events

A ***serious AE*** is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (defined as an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or causes prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- results in the development of drug dependency or drug abuse,

- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.) **For reporting purposes, also consider the occurrences of pregnancy or overdose (regardless of adverse outcome) as events which must be reported as important medical events.**
- An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important. For reporting purposes, Bristol-Myers-Squibb and Boehringer-Ingelheim consider an overdose, regardless of adverse outcome, as an important medical event (see Serious Adverse Events).

All AEs that do not meet any of the criteria for serious should be regarded as nonserious AEs.

The terms “severe” and “serious” are not synonymous. Severity (or intensity) refers to the grade of a specific AE, e.g., mild (Grade 1), moderate (Grade 2), or severe (Grade 3) myocardial infarction (see Section 9.6). “Serious” is a regulatory definition (see previous definition) and is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient’s life or functioning. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations from the Sponsor to applicable regulatory authorities.

Severity and seriousness should be independently assessed when recording AEs and SAEs on the CRF. All serious AEs whether related or unrelated to investigational product, must be immediately reported to Bristol-Myers Squibb (or designee) and Boehringer-Ingelheim by confirmed facsimile transmission to comply with regulatory requirements. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

9.2 Methods and Timing for Assessing AND Recording Safety variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, are recorded on the CRF and reported to the Sponsor in accordance with protocol instructions.

Death as a result of disease progression endpoints are only to be assessed as efficacy measures and not as AEs or SAEs.

9.2.1 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. In addition, the investigator should notify Boehringer and BMS of any SAE that may occur after this time period which they believe to be certainly, probably or possibly related to investigational product.

SAEs that are observed or reported prior to initiation of study treatment should be recorded as SAEs on the CRF if they are associated with protocol-mandated interventions (e.g., invasive procedures such as biopsies, medication washout, or no treatment run-in).

9.2.2 Assessment of Adverse Events

Investigators will assess the occurrence of AEs and SAEs at all patient evaluation timepoints during the study. All AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be recorded in the patient's medical record and on the appropriate AE or SAE CRF page.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity (see Table 11), regulatory seriousness criteria if applicable, suspected relationship to the investigational product (see following guidance), and actions taken.

The AE grading (severity) scale found in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 4.0, will be used for AE reporting. The table below is provided for reference purposes only. In the advent of any inconclusive or discordant findings, the current CTCAE Version 4.0 holds precedence.

Table 11: Adverse Event Grading (Severity) Scale

Grade	*Severity*	*Alternate Description* [a]
1	*Mild (apply event-specific NCI-CTCAE grading criteria)*	*Transient or mild discomfort (<48 hours); no interference with the patient's daily activities; no medical intervention/therapy required*
2	*Moderate (apply event-specific NCI-CTCAE grading criteria)*	*Mild to moderate interference with the patient's daily activities; no or minimal medical intervention/therapy required*
3	*Severe (apply event-specific NCI-CTCAE grading criteria)*	*Considerable interference with the patient's daily activities; medical intervention/therapy required; hospitalization possible*
4	*Very severe, life threatening, or disabling (apply event-specific NCI-CTCAE grading criteria)*	*Extreme limitation in activity; significant medical intervention/therapy required, hospitalization probable*
5	*Death related to AE*	

Note: Regardless of severity, some events may also meet regulatory serious criteria. Refer to definitions of an SAE (see Section 9.3).

[a] *Use these alternative definitions for Grade 1, 2, 3, and 4 events when the observed or reported AE is not in the NCI-CTCAE listing.*

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

- **YES**

  There is a plausible temporal relationship between the onset of the AE and administration of the investigational product, and the AE cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the investigational product; and/or the AE abates or resolves upon discontinuation of the investigational product or dose reduction and, if applicable, reappears upon re-challenge.

- **NO**

  Evidence exists that the AE has an etiology other than the investigational product (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to administration of the investigational product (e.g., cancer diagnosed 2 days after first dose of study drug).

9.2.3 Data Safety and Monitoring Board

The designated cancer center Data Safety and Monitoring Committee (DSMC) will perform reviews of reported adverse events and investigator compliance with adverse event tracking. The DSMC includes physician representation from each program area and a biostatistician. The DSMC is authorized to suspend a trial for non-compliance or as a result of audit findings deemed unacceptable. The DSMC will report significant findings to the IRB, the sponsor, and the applicable regulatory body. Interim meetings are scheduled to address specific issues that require immediate attention to ensure safety of research participants.

9.3 Adverse Event Reporting Guidelines

9.3.1 MedWatch 3500A Reporting for Good Clinical Practice

Unexpected, drug-related SAEs should be reported on the MedWatch Form 3500A, which can be accessed at:

http://www.accessdata.fda.gov/scripts/MedWatch/

MedWatch forms should be sent to the FDA online at the above internet address or at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)

9.3.2 Responsibilities for SAE reporting to Collaborators

All SAEs should simultaneously be faxed to Bristol-Myers Squibb and Boehringer-Ingelheim at:

Global Pharmacovigilance
Bristol-Myers Squibb Company
Fax Number: 609-818-3804

Boehringer-Ingelheim Safety reporting
SAE form should be faxed to BI drug safety unique entry point:

Boehringer Inglehiem Pharmaceuticals, Inc
Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Road
Ridgefield, CT 06877

Fax: 1-203-837-4329
E-mail: casefile.rdg@boehringer-ingelheim.com

If no confirmation of receipt received from BI within 1 working day, the investigator must resend SAE report.

The investigator shall report all SAEs and non-serious AEs occurring at the same time which are medically related to the SAE by fax or other secure method to the BI Unique Entry Point in accordance with the following timelines:

- within five (5) calendar days upon receipt of initial and follow-up SAEs containing at least one fatal or immediately life-threatening event;
- within ten (10) calendar days upon receipt of any other initial and follow-up SAEs.

Each initial and follow-up SAE report form shall be provided with a coversheet form (provided by BI). The coversheet form shall be completely filled out and indicate whether or not the reported SAE(s) qualifies for expedited submission to the competent regulatory authorities, IRB/IECs and/or investigators, if applicable.
The SAE report form shall include all SAEs and non-serious AEs occurring in the same time which are medically related and, in particular, the following information:
Relevance of the reported events based on BI Investigator's Brochure for the Study Drug, Sponsor's causal assessment as to whether the event(s) is/are related to the use of the Study Drug, and the seriousness of each AE.

Occasionally, Bristol-Myers Squibb or Boehringer-Ingelheim may contact the reporter for additional information, clarification or current status of the subject for whom an adverse event was reported.

9.4 Procedures for Eliciting, Recording, and Reporting Adverse Events

Eliciting Adverse Events:

- A consistent methodology of non-directive questioning for eliciting AEs at all patient evaluation timepoints should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"

- "Tell me about any new or changed health problems since you were last here?"

9.4.1 Expedited Reporting Requirements for Serious Adverse Events

Investigators will submit written reports of all SAEs, regardless of attribution, to H. Lee Moffitt Internal Monitoring Commitee (DSMC) within 48 hours, respectively, of learning of the events via OnCore ®. For initial SAE reports, investigators should record all case details that can be gathered within 48 hours on an SAE CRF page.

9.4.2 Type and Duration of Follow-up of Patients after Adverse Events

Collection of complete information concerning AEs and SAEs is extremely important. All AEs and SAEs that are encountered during the protocol-specified AE reporting period should be followed to their resolutions, or until the investigator assesses them as stable, or the patient is lost to follow-up. Resolution of AEs and SAEs (with dates) should be documented on the appropriate AE or SAE CRF page in OnCore ® and in the patient's medical record to facilitate source data verification.

For some SAEs, the Sponsor or its designee may follow-up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

10 [redacted]

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11 ADMINISTRATIVE SECTION

Protection of Human Subjects from Research Risk:

11.1 Risk to Subjects

The risk to subjects will be outlined clearly and in detail in the informed consent. The collection of peripheral venous blood for study purposes is not associated with an additional risk to participants since it will be collected during routine phlebotomy, which is required for monitoring before and while on chemotherapy.

11.2 Adequacy of Protection Against Risk

To protect participants from excess risk, the above-mentioned study procedures and dose-escalation scheme were instituted. Additional protection is provided through the data safety and monitoring plan described below.

11.3 Importance of the Knowledge to be Gained

The development of a well-tolerated and effective regimen in a disease could potentially at worst add to the armamentarium of available regimens and at best change standard of care. Specific strategies to improve the care of patients relapsing following chemotherapy for lung cancer are direly needed.

11.4 Data Safety and Monitoring Plan

The Data Safety & Monitoring Plan (DSMP) will ensure that this trial is well designed, responsibly managed, appropriately reported, and that it protects the rights and welfare of patients. The following internal and external review and monitoring processes provide oversight and active monitoring of this trial:

The Principal Investigators (PI)

The Clinical Trials Office (CTO)

The Scientific Review Committee (SRC)

The Protocol Review and Monitoring Committee (PRMC) (aka DSMC);

The Research Compliance Division (RCD) of the Cancer Center's Compliance Office;

The University of South Florida, Institutional Review Board (USF IRB).

The protocol includes a section that specifies the following with respect to Adverse Event reporting: what constitutes an adverse event (versus what is a serious adverse event), the

entities to which adverse events should be reported, the timing of this reporting, and the person or persons responsible for reporting. This includes prompt (within one day of knowledge of the event) reporting to the IRB for unanticipated risks to subjects and reporting in writing within five working days to the IRB and sponsor.

11.5 Initial and Ongoing Monitoring and Review

Principal Investigator: The PI of the study has primary responsibility for ensuring that the protocol is conducted as approved by the PRMC and the IRB. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to the Scientific Review Committee (SRC), Protocol Review and Monitoring Committee (PRMC) and IRB as required, and that all adverse events are appropriately reported.

Clinical Trials Office: The CTO will provide support for the monitoring of this trial, and these functions consist of the following:

a) Data management by a research coordinator whose primary functions include:

1. Supporting the PI in screening and enrolling patients onto the trial

2. Providing data management support

3. Providing staff education and training

4. Coordinating and implementing protocol-related orders for study patients

Preparing medical charts for audits

b) Regulatory support by specialists trained in both the Federal regulations and USF Institutional Review Board policies. The specialists' primary functions include:

1. Assistance in the preparation and writing of the clinical protocol

2. Assistance in writing of the informed consent

3. Coordination and facilitation of communication to research oversight committees

4. Assistance in submission of regulatory documents such as FDA 1572s, NIH Annual Reporting, adverse event reports and accrual reports

5. Coordination of clinical trial activation and closure

6. Maintenance of institutional clinical research regulatory documents

The Scientific Review Committee

The Cancer Center's internal Scientific Review Committee (SRC) provides for a formal internal peer review of all protocols and general scientific oversight of interventional clinical research. The Committee has a defined membership representing all of the major research divisions of the Cancer Center, including biostatisticians. All new protocol submissions must contain the required elements of the protocol, and must include a DSMP prior to approval by the Committee. The plan has to be appropriate for the phase and risk of the proposed study. Two formal reviewers are pre-assigned to lead discussion of the protocol. The committee's vote indicates approval, conditional approval, or disapproval. Approval by majority vote is required. Members affiliated with the protocol must leave the room during the discussion and voting process. SRC approval is required before the protocol can be submitted to the IRB.

The Protocol Review and Monitoring Committee

The Protocol Review and Monitoring Committee (PRMC) will monitor this trial for safety, progress, protocol compliance, accrual, adverse event reporting, and data integrity. The membership of the PRMC includes physician representation from each program area and a biostatistician. In addition to the existing stopping rules, the PRMC is authorized to suspend a trial for non-compliance with a DSMP or as a result of audit findings deemed unacceptable. The PRMC will report significant findings to the IRB, the sponsor, and the applicable regulatory body. Interim meetings are scheduled to address specific issues that require immediate attention to ensure safety of research participants.

Internal Monitoring

Data will be captured in Oncore, Moffitt's Clinical Trials Database. The Case Report Forms will be reviewed by Moffitt's Internal Monitors, periodically, throughout the conduct of the trial. The monitoring will include source data verification, utilizing research subjects' medical records.The Research Compliance Division of the Cancer Center's Compliance Office

The Cancer Center has established the Research Compliance Division (RCD) as the coordinating office for internal audits of all clinical trials conducted at the Cancer Center and its affiliates.

The purpose of the internal audit program is to:

1. Assure patient safety by monitoring compliance

2. Assure regulatory compliance by reviewing consent and adverse event reporting

3. Assure scientific value by monitoring accuracy and completeness of data collection

4. Monitor and coordinate research compliance activities associated with institutional and individual conflict of interest

5. Make recommendations for modification of research practices as necessary and provide education on issues that are critical to good research practices

Audits will be conducted by the RCD in accordance with applicable regulatory standards. Investigator initiated trials, such as the one proposed here, receive the highest priority for audit. The RCD will conduct and report the findings of audits to the PMC in accordance with a protocol's annual review. The PRMC will determine the findings to be acceptable with minor deviations, acceptable with corrective action, or unacceptable with suspension or closure. The PRMC Chairperson will notify the IRB of the audit findings. The RCD will follow-up to ascertain whether corrective actions, which have been agreed to, are achieving the desired results. The PRMC will be informed of all significant open follow-up items. For those observations where no action has been taken, the Research Compliance Office will inform the PRMC and may conduct a focused audit.

The University of South Florida Institutional Review Board

The University of South Florida Institutional Review Board (USF IRB), the IRB contracted by The Cancer Center, must review and approve all clinical research protocols prior to activation. The DSMP constitutes a significant element of this review.

11.6 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50).

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion before initiation of the study.

All potential serious breaches must be reported to Boehringer-Ingelheim (BI) and Bristol Myers Squibb (BMS) immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant

degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (eg, loss of medical licensure; debarment). Systems with procedures that ensure the quality of every aspect of the study will be implemented.

11.7 Suspension and Termination

The PRMC and or the IRB may vote to suspend or terminate approval of this trial if on review it is determined that the trial is not being conducted in accordance with the IRB, Cancer Center, and/or regulatory requirements or that it has been associated with unexpected problems or serious harm to subjects. The PRMC/IRB will notify the PI in writing of such suspensions or terminations. It is the responsibility of the PRMC/IRB chairperson to ensure prompt written notification of any suspensions or termination of PRMC/IRB approval to the relevant Federal Agencies, including OHRP, FDA, and the study sponsor/funding source.

11.8 Institutional and Individual Conflicts of Interest

Conflict of interest (COI) is defined as any situation in which financial or personal obligations may compromise or present the appearance of compromising an individual's or group's professional judgment in conducting, reviewing or reporting research. This includes research personnel, IRB members, research administration officials, the Cancer Center, the University of South Florida, and research sponsors.

The processes for managing individual and institutional COI will focus on disclosure of the conflict, managing the conflict, and prohibiting the activity when necessary to protect the public interest or the interest of the Cancer Center.

Prior to the activation of this trial and ongoing thereafter, individuals who are involved in the design, conduct, and reporting will be required to disclose to the SRC any and all financial or other interests that are, or may perceived to be, related to the trial in which they are actively involved in. The SRC will determine if a conflict exists, and if so, assure that the COI is managed, reduced or eliminated. This information will accompany a protocol when it is presented to the IRB. The IRB will retain the highest authority for determining that the conflict of interest has been properly disclosed, managed, or eliminated. The PRMC will ensure ongoing monitoring of conflict of interest through monthly review of conflict of interest disclosures. If a COI exists, the PMC will ensure that a plan to manage, reduce or eliminate

the COI is developed and submitted to the IRB for approval and the protocol is amended accordingly. The RCD will be responsible for maintaining a secure data system for tracking and timely reporting of disclosure information to the SRC or PRMC for review as appropriate. The RCD will be responsible for ensuring coordination and communication of COI between the Cancer Center and the IRB. The IRB has the final authority to determine that the COI has been managed sufficiently to assure the protection of human participants.

11.9 Records Retention

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

11.10 Contributions

Dr. Takeshi Yoshida, a post-doctoral fellow in the Haura Lab, has contributed to this protocol by helping to write the background portion of the Introduction and contribution to preclinical studies.

Drs. Patricia LoRusso, Gary M Clark, and Miguel Villalona have contributed to this protocol by revision and editing.

Barbara LeStage, a patient advocate, has contributed to this protocol in design and editing.

Angela Reagan has contributed to revision and editing of this protocol.

Rasa Hamilton has contributed to editing of this protocol.

Moffitt LATTE (Lung And Thoracic Tumors Education) Advocate advisory panel has contributed to the design and review of this protocol.

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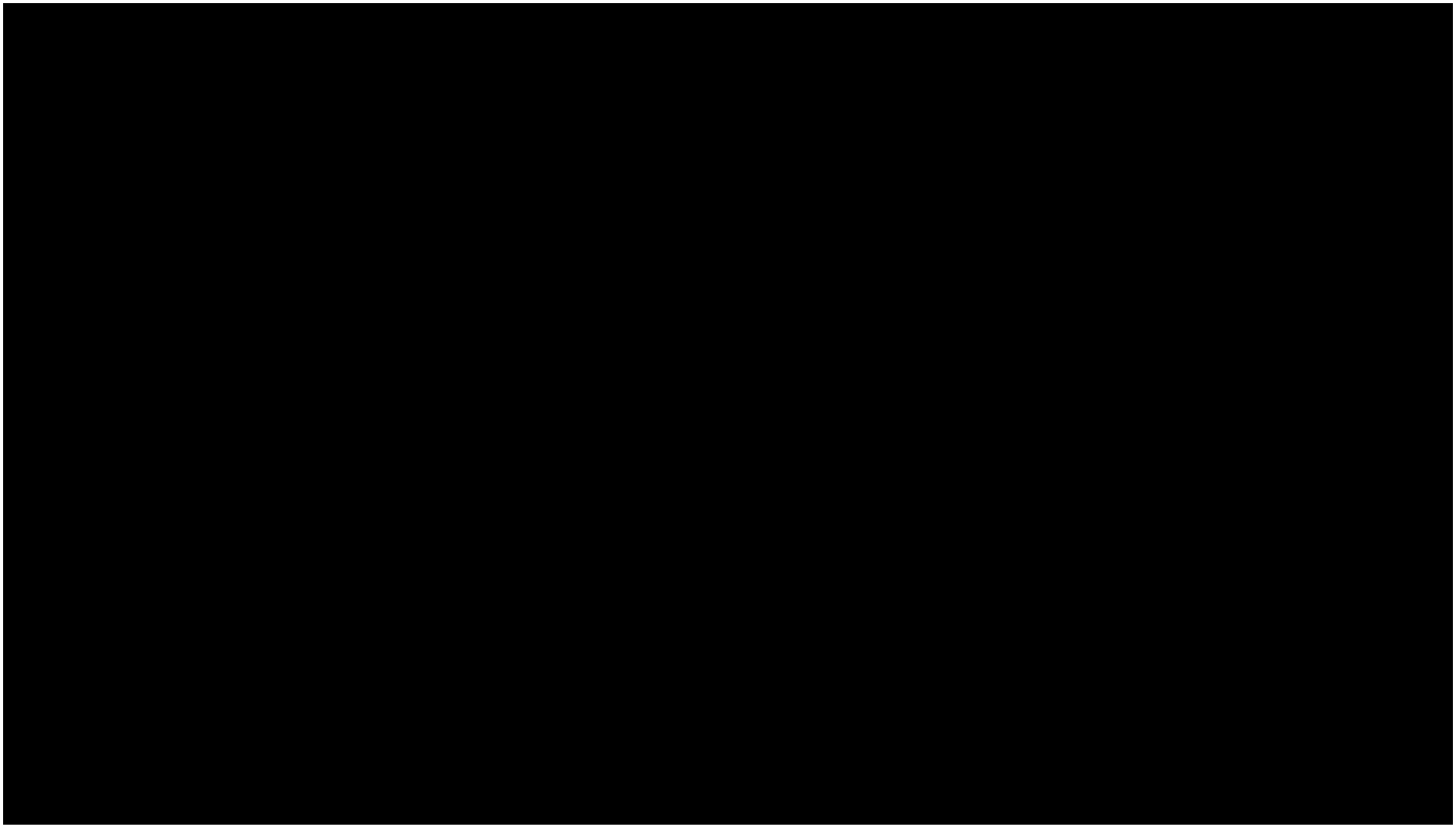

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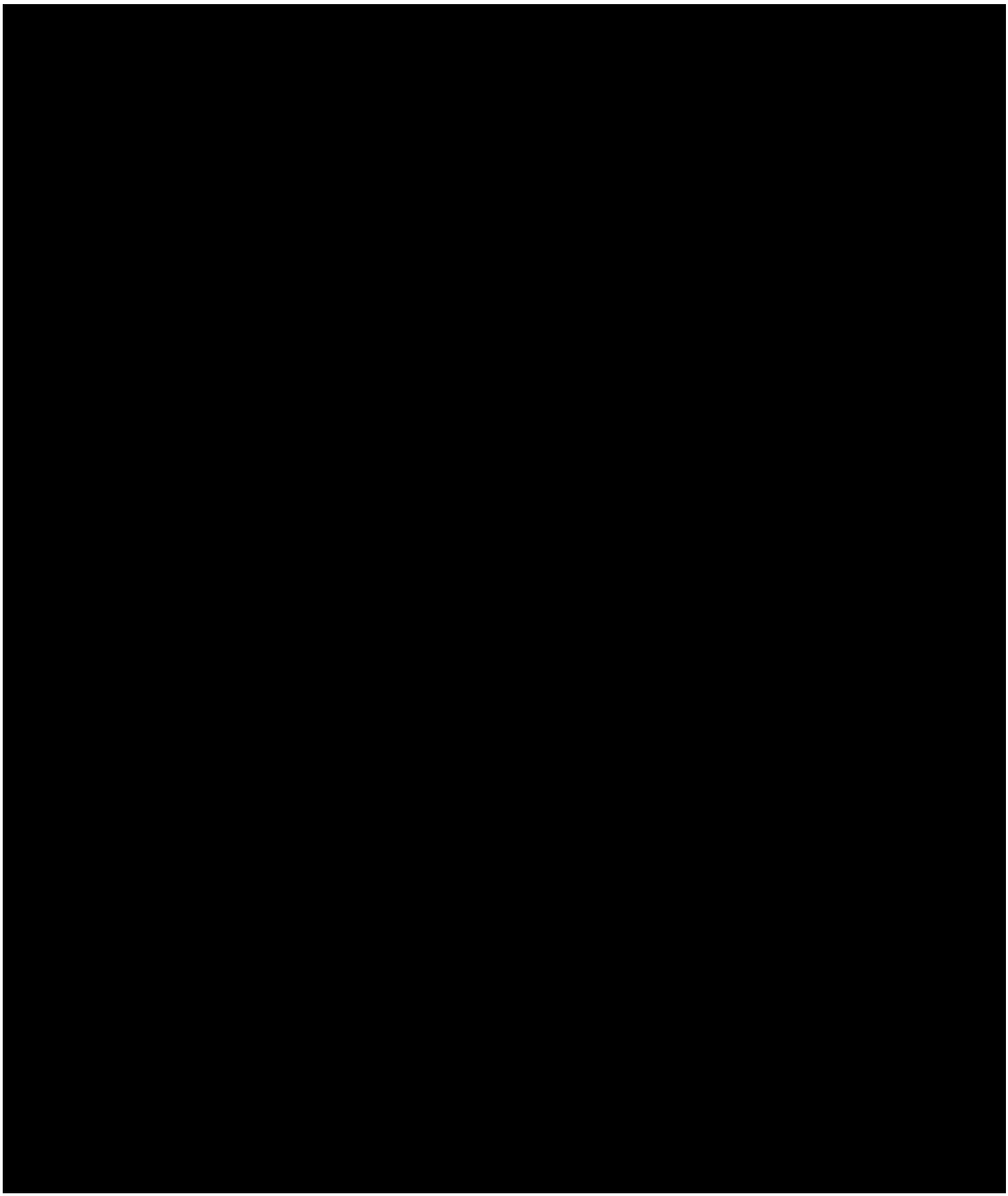

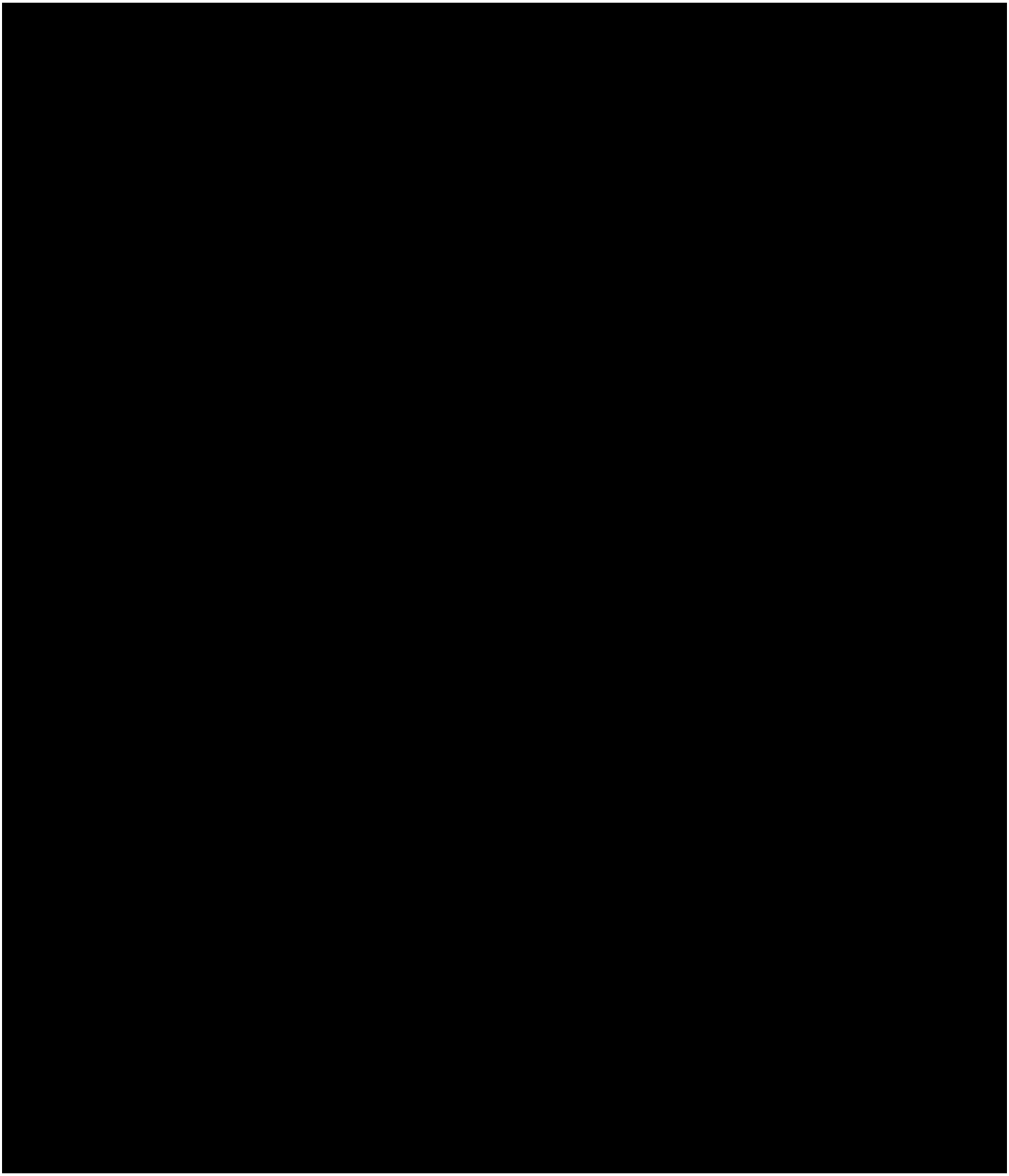

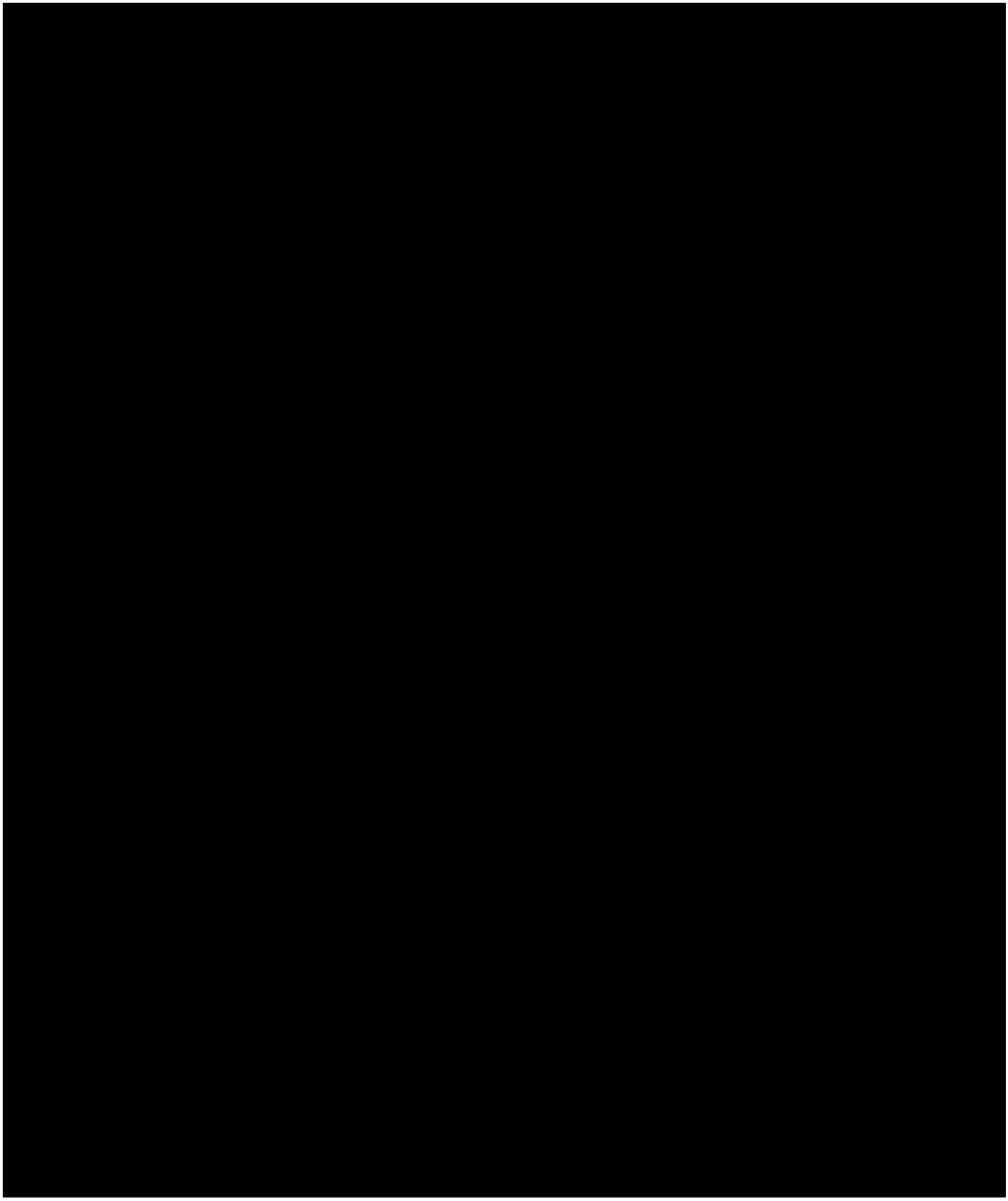

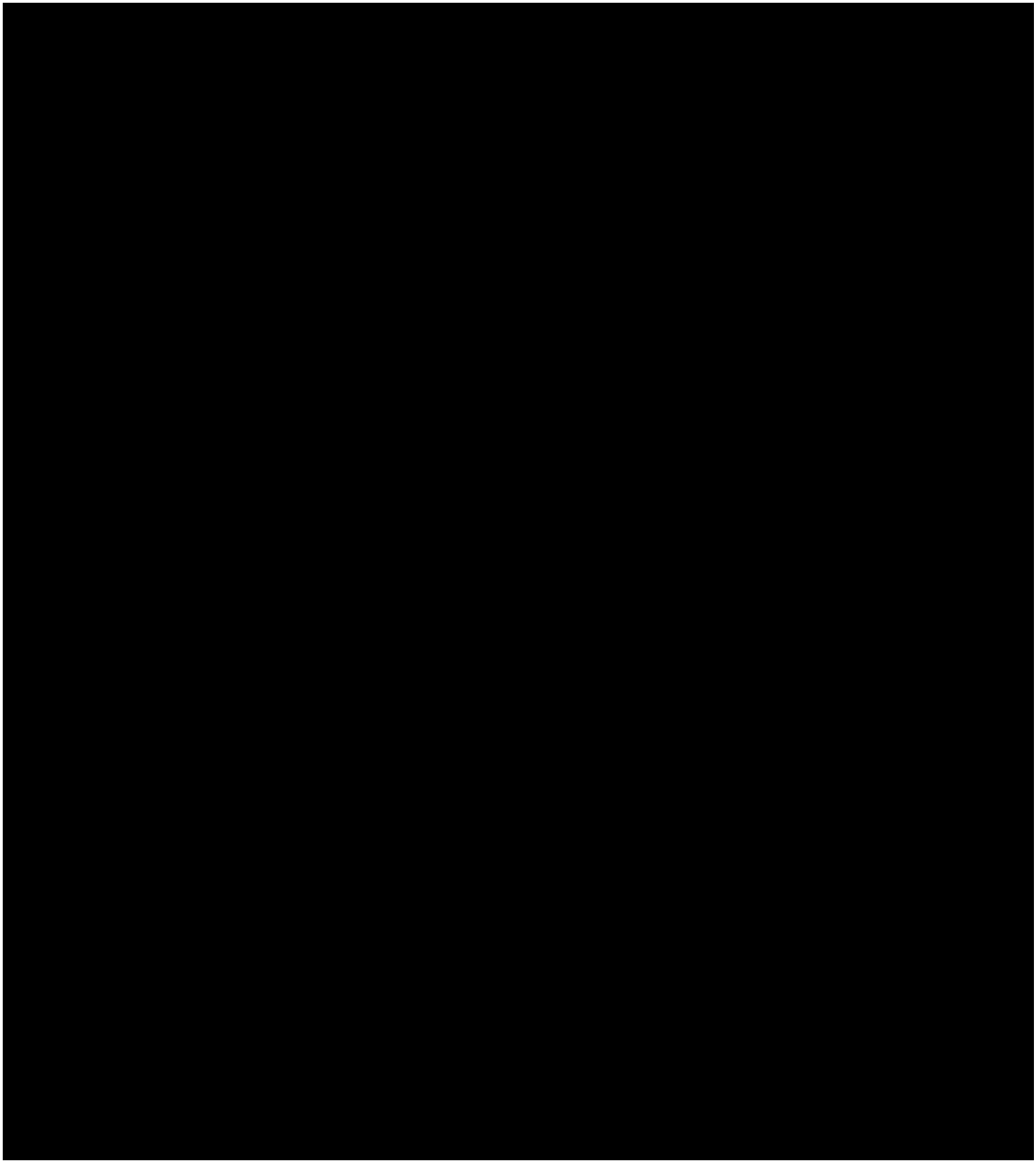

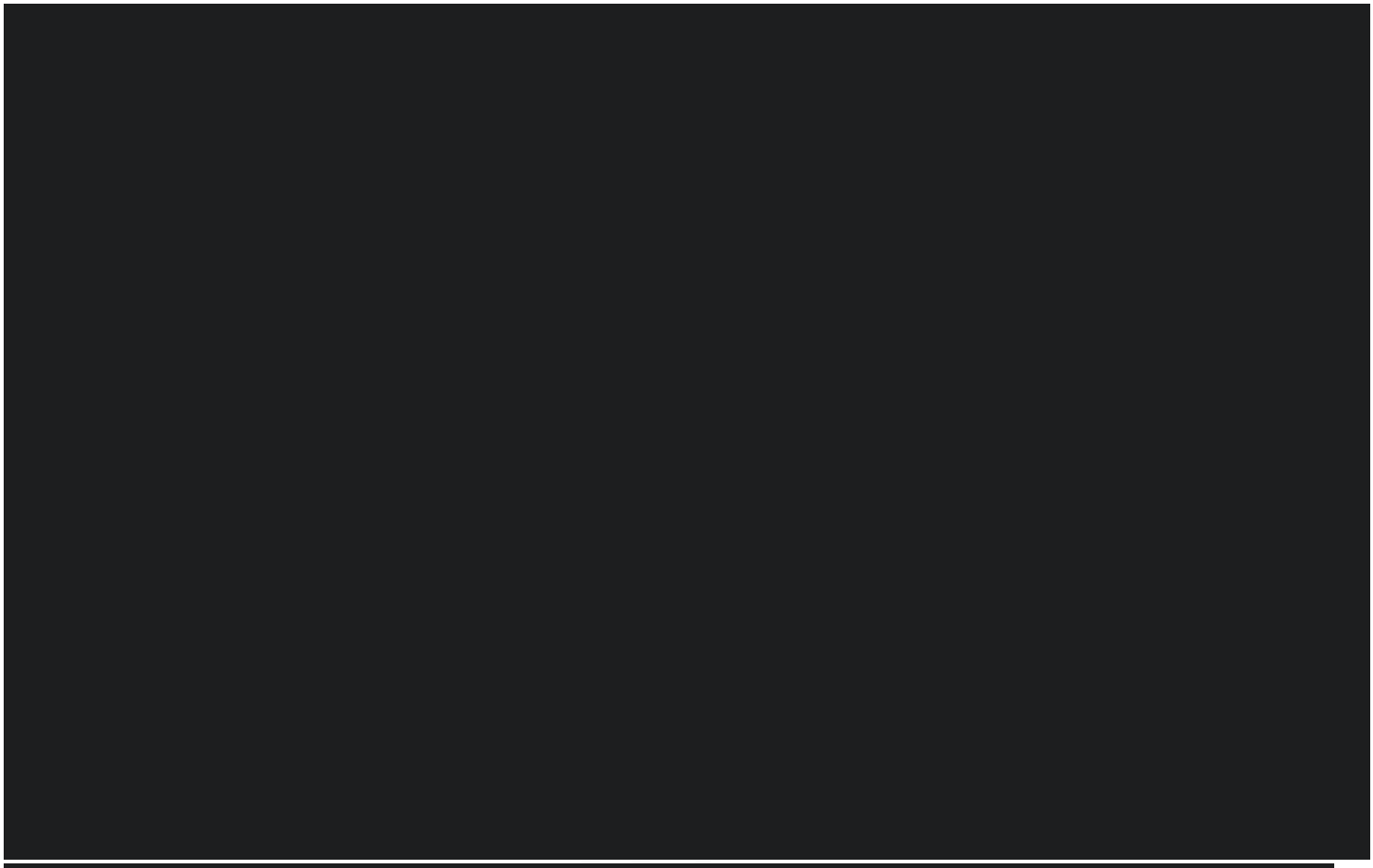

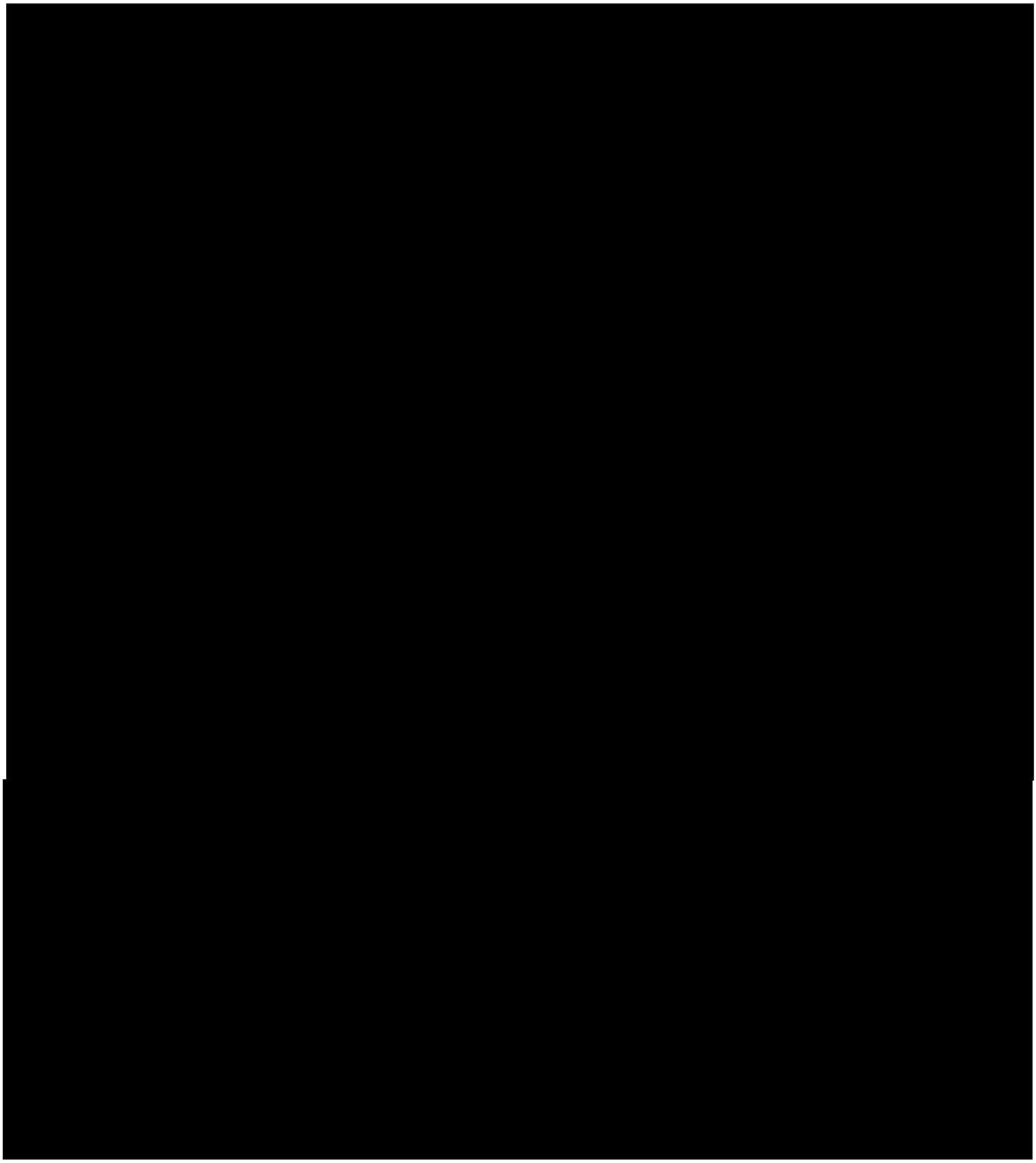

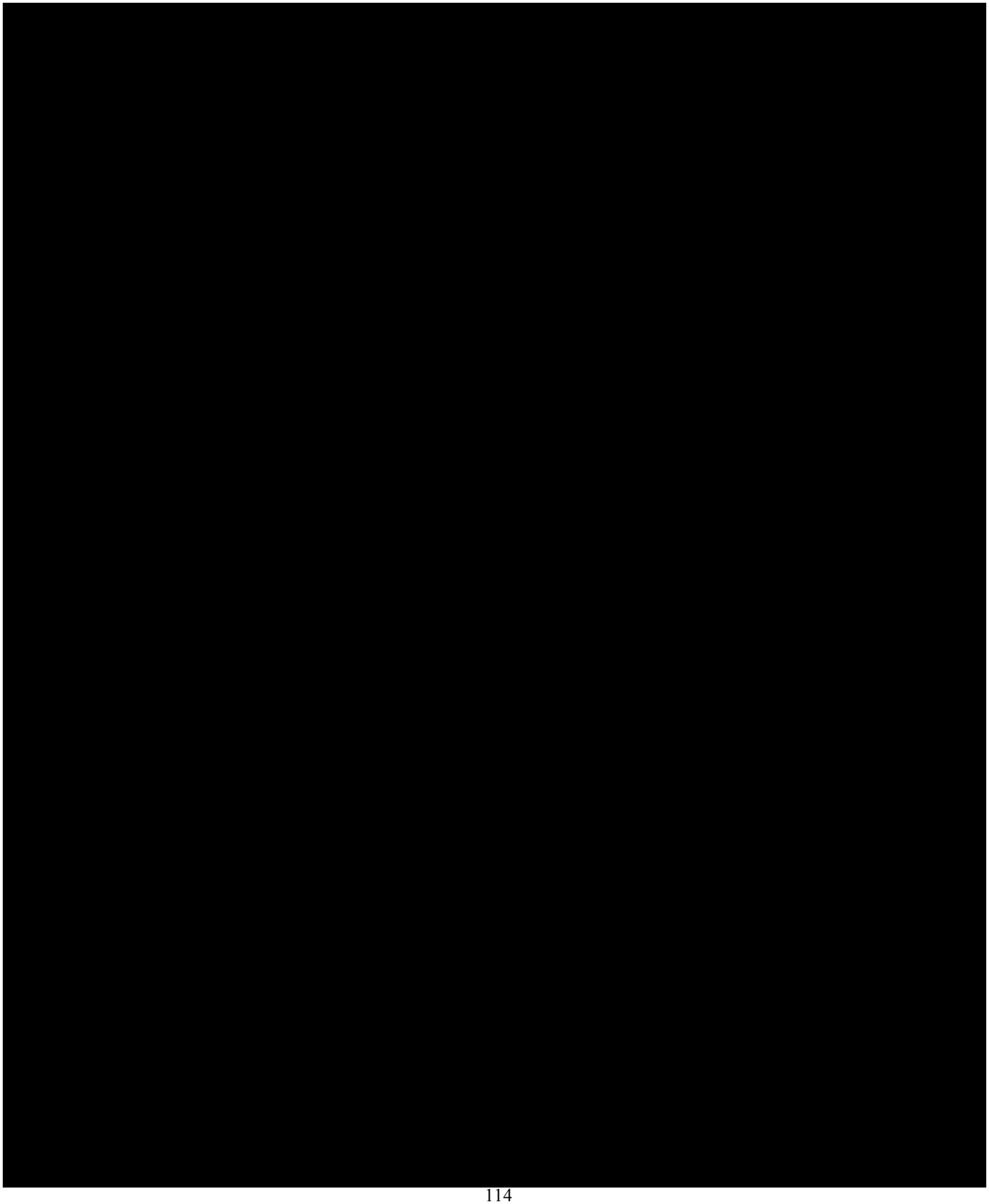

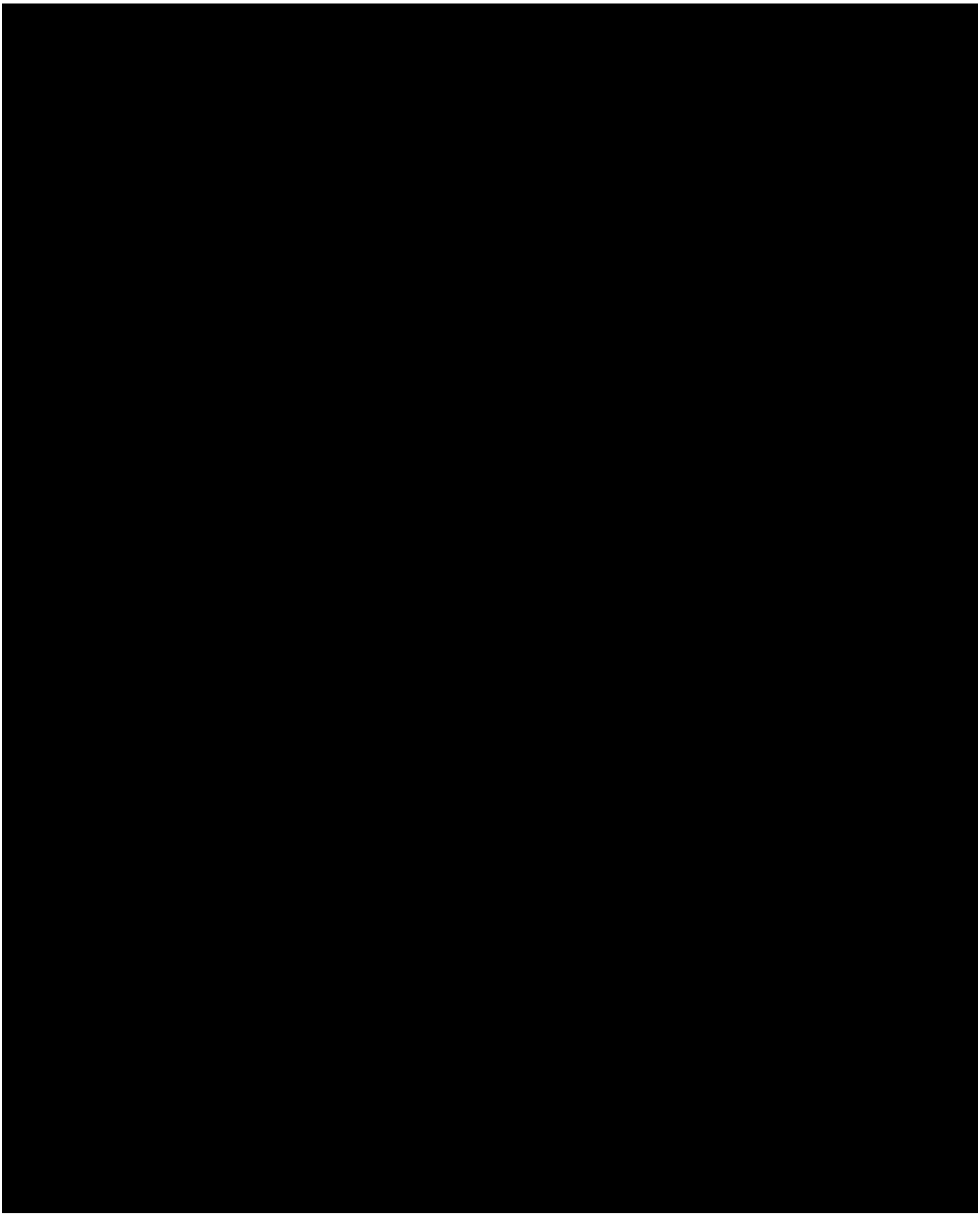

13 LIST OF ABBREVIATIONS

AE	Adverse event
ANC	Absolute Neutrophil Count
BID	Twice a Day
BI	Boehringer-Ingelheim Company
BMS	Bristol-Myers Squibb Company
CAT (or CT scan)	Computed Axial Tomography
CBC	Complete Blood Count
CR	Complete Response
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMC	Data Safety Monitoring Committee
ECOG PS	Eastern Cooperative Oncology Group Performance Status
EKG	Electrocardiogram
EGFR	Epidermal Growth Factor Receptor
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practice

HCG	Human Chorionic Gonadotropin
HRT	Hormone Replacement Therapy
IB	Investigators' Brochure
ICH	International Conference on Harmonisation
IND	Investigational New Drug (Application)
IRB	Institutional Review Board
IIS	Investigator-Initiated Trial
MTD	Maximum Tolerated Dose
MAD	Maximum Administered Dose
MRI	Magnetic Resonance Imaging
PD	Progressive Disease
PFS	Progression Free Survival
PO	By Mouth
PRMC	Protocol Review & Monitoring Committee
PR	Partial Response
QD	Once Daily
QoL	Quality Of Life
RECIST	Response Evaluation Criteria In Solid Tumors
SAE	Serious Adverse Event
SD	Stable Disease
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNM Staging	Tumor, Node and Metastasis Staging
ULN	Upper Limit of Normal
WBC	White Blood Count
WOCBP	Women of Child-Bearing Potential

14 REFERENCES

1. Crown, J.P., et al., *Pooled analysis of diarrhea events in patients with cancer treated with lapatinib.* Breast cancer research and treatment, 2008. **112**(2): p. 317-25.

2. Masiello, D., G. Gorospe, 3rd, and A.S. Yang, *The occurrence and management of fluid retention associated with TKI therapy in CML, with a focus on dasatinib.* Journal of hematology & oncology, 2009. **2**: p. 46.

3. Jordan, K., C. Sippel, and H.J. Schmoll, *Guidelines for antiemetic treatment of chemotherapy-induced nausea and vomiting: past, present, and future recommendations.* The oncologist, 2007. **12**(9): p. 1143-1150.

4. Joensuu, H., J.C. Trent, and P. Reichardt, *Practical management of tyrosine kinase inhibitor-associated side effects in GIST.* Cancer treatment reviews, 2011. **37**(1): p. 75-88.

5. Balagula, Y., et al., *Clinical presentation and management of dermatological toxicities of epidermal growth factor receptor inhibitors.* International journal of dermatology, 2011. **50**(2): p. 129-46.

6. Wu, P.A., et al., *Prophylaxis and treatment of dermatologic adverse events from epidermal growth factor receptor inhibitors.* Current opinion in oncology, 2011. **23**(4): p. 343-51.

7. Chaft, J.E., et al., *Disease Flare after Tyrosine Kinase Inhibitor Discontinuation in Patients with EGFR-Mutant Lung Cancer and Acquired Resistance to Erlotinib or Gefitinib: Implications for Clinical Trial Design.* Clinical Cancer Research, 2011. **17**(19): p. 6298-6303.

8. Jemal, A., et al., *Cancer statistics, 2010.* CA: a cancer journal for clinicians, 2010. **60**(5): p. 277.

9. *SEER* http://www.seer.cancer.gov 2000 - 2007.

10. Jemal, A., et al., *Cancer statistics, 2009.* CA Cancer J Clin, 2009. **59**(4): p. 225-49.

11. *Non-small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials.* BMJ, 1995. **311**(7010): p. 899-909.

12. Pfister, D.G., et al., *American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003.* J Clin Oncol, 2004. **22**(2): p. 330-53.

13. Socinski, M.A., et al., *Treatment of non-small cell lung cancer, stage IV: ACCP evidence-based clinical practice guidelines (2nd edition).* Chest, 2007. **132**(3 Suppl): p. 277S-289S.

14. Group, N.M.-A.C., *Chemotherapy in addition to supportive care improves survival in advanced non-small-cell lung cancer: a systematic review and meta-analysis of individual patient data from 16 randomized controlled trials.* J Clin Oncol, 2008. **26**(28): p. 4617-25.

15. Shepherd, F.A., et al., *Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy.* J Clin Oncol, 2000. **18**(10): p. 2095-103.

16. Noble, J., et al., *Second-line or subsequent systemic therapy for recurrent or progressive non-small cell lung cancer: a systematic review and practice guideline.* J Thorac Oncol, 2006. **1**(9): p. 1042-58.

17. Hirsch, F.R., et al., *Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis.* J Clin Oncol, 2003. **21**(20): p. 3798-807.

18. Suzuki, S., et al., *Protein overexpression and gene amplification of epidermal growth factor receptor in nonsmall cell lung carcinomas. An immunohistochemical and fluorescence in situ hybridization study.* Cancer, 2005. **103**(6): p. 1265-73.

19. Carpenter, G., *Receptors for epidermal growth factor and other polypeptide mitogens.* Annu Rev Biochem, 1987. **56**: p. 881-914.

20. Riese, D.J., 2nd and D.F. Stern, *Specificity within the EGF family/ErbB receptor family signaling network.* Bioessays, 1998. **20**(1): p. 41-8.

21. Ogiso, H., et al., *Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains.* Cell, 2002. **110**(6): p. 775-87.

22. Schlessinger, J., *Ligand-induced, receptor-mediated dimerization and activation of EGF receptor.* Cell, 2002. **110**(6): p. 669-72.

23. Di Marco, E., et al., *Autocrine interaction between TGF alpha and the EGF-receptor: quantitative requirements for induction of the malignant phenotype.* Oncogene, 1989. **4**(7): p. 831-8.

24. Klapper, L.N., et al., *Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors.* Adv Cancer Res, 2000. **77**: p. 25-79.

25. Normanno, N., et al., *Epidermal growth factor receptor (EGFR) signaling in cancer.* Gene, 2006. **366**(1): p. 2-16.

26. Scaltriti, M. and J. Baselga, *The epidermal growth factor receptor pathway: a model for targeted therapy.* Clin Cancer Res, 2006. **12**(18): p. 5268-72.

27. Vieira, A.V., C. Lamaze, and S.L. Schmid, *Control of EGF receptor signaling by clathrin-mediated endocytosis.* Science, 1996. **274**(5295): p. 2086-9.

28. Sorkin, A., *Internalization of the epidermal growth factor receptor: role in signalling.* Biochem Soc Trans, 2001. **29**(Pt 4): p. 480-4.

29. Wang, Y., et al., *Endosomal signaling of epidermal growth factor receptor stimulates signal transduction pathways leading to cell survival.* Mol Cell Biol, 2002. **22**(20): p. 7279-90.

30. Yun, C.H., et al., *Structures of lung cancer-derived EGFR mutants and inhibitor complexes: mechanism of activation and insights into differential inhibitor sensitivity.* Cancer Cell, 2007. **11**(3): p. 217-27.

31. Kumar, A., et al., *Structure and clinical relevance of the epidermal growth factor receptor in human cancer.* J Clin Oncol, 2008. **26**(10): p. 1742-51.

32. Fukuoka, M., et al., *Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected].* J Clin Oncol, 2003. **21**(12): p. 2237-46.

33. Kris, M.G., et al., *Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial.* JAMA, 2003. **290**(16): p. 2149-58.

34. Shepherd, F.A., et al., *Erlotinib in previously treated non-small-cell lung cancer.* N Engl J Med, 2005. **353**(2): p. 123-32.

35. Wheatley-Price, P., et al., *Erlotinib for advanced non-small-cell lung cancer in the elderly: an analysis of the National Cancer Institute of Canada Clinical Trials Group Study BR.21.* J Clin Oncol, 2008. **26**(14): p. 2350-7.

36. Lynch, T.J., et al., *Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib.* N Engl J Med, 2004. **350**(21): p. 2129-39.

37. Paez, J.G., et al., *EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy.* Science, 2004. **304**(5676): p. 1497-500.

38. Pao, W., et al., *EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib.* Proc Natl Acad Sci U S A, 2004. **101**(36): p. 13306-11.

39. Murray, S., et al., *Somatic mutations of the tyrosine kinase domain of epidermal growth factor receptor and tyrosine kinase inhibitor response to TKIs in non-small cell lung cancer: an analytical database.* J Thorac Oncol, 2008. **3**(8): p. 832-9.

40. Rosell, R., et al., *Screening for epidermal growth factor receptor mutations in lung cancer.* N Engl J Med, 2009. **361**(10): p. 958-67.

41. Tanaka, T., et al., *Frequency of and variables associated with the EGFR mutation and its subtypes.* Int J Cancer 2010;. **126**(3): p. 651-5.

42. Mulloy, R., et al., *Epidermal growth factor receptor mutants from human lung cancers exhibit enhanced catalytic activity and increased sensitivity to gefitinib.* Cancer Res, 2007. **67**(5): p. 2325-30.

43. Okabe, T., et al., *Differential constitutive activation of the epidermal growth factor receptor in non-small cell lung cancer cells bearing EGFR gene mutation and amplification.* Cancer Res, 2007. **67**(5): p. 2046-53.

44. Choi, S.H., J.M. Mendrola, and M.A. Lemmon, *EGF-independent activation of cell-surface EGF receptors harboring mutations found in gefitinib-sensitive lung cancer.* Oncogene, 2007. **26**(11): p. 1567-76.

45. Chen, Y.R., et al., *Distinctive activation patterns in constitutively active and gefitinib-sensitive EGFR mutants.* Oncogene, 2006. **25**(8): p. 1205-15.

46. Mitsudomi, T., et al., *Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial.* Lancet Oncol, 2009.

47. Inoue, A., et al., *9LBA A randomized phase III study comparing gefitinib with carboplatin (CBDCA) plus paclitaxel (TXL) for the first-line treatment of non-small cell lung cancer (NSCLC) with sensitive EGFR mutations: NEJ002 study.* European Journal of Cancer Supplements, 2009. **7**(3): p. 6-6.

48. Mok, T.S., et al., *Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma.* N Engl J Med, 2009. **361**(10): p. 947-57.

49. Fukuoka, M., et al., *Biomarker analyses from a phase III, randomized, open-label, first-line study of gefitinib (G) versus carboplatin/paclitaxel (C/P) in clinically selected patients (pts) with advanced non-small cell lung cancer (NSCLC) in Asia (IPASS).* J Clin Oncol (Meeting Abstracts), 2009. **27**(15S): p. 8006-.

50. Kobayashi, S., et al., *EGFR mutation and resistance of non-small-cell lung cancer to gefitinib.* N Engl J Med, 2005. **352**(8): p. 786-92.

51. Pao, W., et al., *Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain.* PLoS Med, 2005. **2**(3): p. e73.

52. Balak, M.N., et al., *Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors.* Clin Cancer Res, 2006. **12**(21): p. 6494-501.

53. Kosaka, T., et al., *Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib.* Clin Cancer Res, 2006. **12**(19): p. 5764-9.

54. Stamos, J., M.X. Sliwkowski, and C. Eigenbrot, *Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor.* J Biol Chem, 2002. **277**(48): p. 46265-72.

55. Clark, J., J. Cools, and D.G. Gilliland, *EGFR inhibition in non-small cell lung cancer: resistance, once again, rears its ugly head.* PLoS Med, 2005. **2**(3): p. e75.

56. Godin-Heymann, N., et al., *Oncogenic activity of epidermal growth factor receptor kinase mutant alleles is enhanced by the T790M drug resistance mutation.* Cancer Res, 2007. **67**(15): p. 7319-26.

57. Yun, C.H., et al., *The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP.* Proc Natl Acad Sci U S A, 2008. **105**(6): p. 2070-5.

58. Kobayashi, S., et al., *An alternative inhibitor overcomes resistance caused by a mutation of the epidermal growth factor receptor.* Cancer Res, 2005. **65**(16): p. 7096-101.

59. Engelman, J.A., et al., *Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer.* J Clin Invest, 2006. **116**(10): p. 2695-706.

60. Engelman, J.A., et al., *PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib.* Cancer Res, 2007. **67**(24): p. 11924-32.

61. Gonzales, A.J., et al., *Antitumor activity and pharmacokinetic properties of PF-00299804, a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor.* Mol Cancer Ther, 2008. **7**(7): p. 1880-9.

62. Li, D., et al., *BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models.* Oncogene, 2008. **27**(34): p. 4702-11.

63. Kwak, E.L., et al., *Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib.* Proc Natl Acad Sci U S A, 2005. **102**(21): p. 7665-70.

64. Sos, M.L., et al., *Chemogenomic Profiling Provides Insights into the Limited Activity of Irreversible EGFR Inhibitors in Tumor Cells Expressing the T790M EGFR Resistance Mutation.* Cancer Res 2010;. **70**(3): p. 868-874.

65. Yu, Z., et al., *Resistance to an irreversible epidermal growth factor receptor (EGFR) inhibitor in EGFR-mutant lung cancer reveals novel treatment strategies.* Cancer Res, 2007. **67**(21): p. 10417-27.

66. Godin-Heymann, N., et al., *The T790M "gatekeeper" mutation in EGFR mediates resistance to low concentrations of an irreversible EGFR inhibitor.* Mol Cancer Ther, 2008. **7**(4): p. 874-9.

67. Shimamura, T., et al., *Hsp90 inhibition suppresses mutant EGFR-T790M signaling and overcomes kinase inhibitor resistance.* Cancer Res, 2008. **68**(14): p. 5827-38.

68. Yamada, T., et al., *Hepatocyte growth factor reduces susceptibility to an irreversible epidermal growth factor receptor inhibitor in EGFR-T790M mutant lung cancer.* Clin Cancer Res, 2010. **16**(1): p. 174-83.

69. Ercan, D., et al., *Amplification of EGFR T790M causes resistance to an irreversible EGFR inhibitor.* Oncogene, 2010. **29**(16): p. 2346-56.

70. Metro, G. and L. Crino, *The LUX-Lung clinical trial program of afatinib for non-small-cell lung cancer.* Expert Rev Anticancer Ther, 2011. **11**(5): p. 673-82.

71. Regales, L., et al., *Dual targeting of EGFR can overcome a major drug resistance mutation in mouse models of EGFR mutant lung cancer.* J Clin Invest, 2009. **119**(10): p. 3000-10.

72. Li, D., et al., *Bronchial and peripheral murine lung carcinomas induced by T790M-L858R mutant EGFR respond to HKI-272 and rapamycin combination therapy.* Cancer Cell, 2007. **12**(1): p. 81-93.

73. Engelman, J.A., et al., *MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling.* Science, 2007. **316**(5827): p. 1039-43.

74. Faber, A.C., et al., *Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition.* Proc Natl Acad Sci U S A, 2009. **106**(46): p. 19503-8.

75. Morgillo, F., et al., *Heterodimerization of insulin-like growth factor receptor/epidermal growth factor receptor and induction of survivin expression counteract the antitumor action of erlotinib.* Cancer Res, 2006. **66**(20): p. 10100-11.

76. Morgillo, F., et al., *Implication of the insulin-like growth factor-IR pathway in the resistance of non-small cell lung cancer cells to treatment with gefitinib.* Clin Cancer Res, 2007. **13**(9): p. 2795-803.

77. Guix, M., et al., *Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins.* J Clin Invest, 2008. **118**(7): p. 2609-19.

78. Sos, M.L., et al., *PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR.* Cancer Res, 2009. **69**(8): p. 3256-61.

79. Yamasaki, F., et al., *Acquired resistance to erlotinib in A-431 epidermoid cancer cells requires down-regulation of MMAC1/PTEN and up-regulation of phosphorylated Akt.* Cancer Res, 2007. **67**(12): p. 5779-88.

80. Yano, S., et al., *Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations.* Cancer Res, 2008. **68**(22): p. 9479-87.

81. Yauch, R.L., et al., *Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients.* Clin Cancer Res, 2005. **11**(24 Pt 1): p. 8686-98.

82. Thomson, S., et al., *Epithelial to mesenchymal transition is a determinant of sensitivity of non-small-cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition.* Cancer Res, 2005. **65**(20): p. 9455-62.

83. Uramoto, H., et al., *Expression of selected gene for acquired drug resistance to EGFR-TKI in lung adenocarcinoma.* Lung Cancer, 2011. **ahead of print**.

84. Yao, Z., et al., *TGF-beta IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer.* Proc Natl Acad Sci U S A, 2010. **107**(35): p. 15535-40.

85. Suda, K., et al., *Epithelial to Mesenchymal Transition in an Epidermal Growth Factor Receptor-Mutant Lung Cancer Cell Line with Acquired Resistance to Erlotinib.* J Thorac Oncol, 2011. **ahead of print**.

86. Kim, L.C., L. Song, and E.B. Haura, *Src kinases as therapeutic targets for cancer.* Nat Rev Clin Oncol, 2009. **6**(10): p. 587-95.

87. Zhang, J., et al., *SRC-family kinases are activated in non-small cell lung cancer and promote the survival of epidermal growth factor receptor-dependent cell lines.* Am J Pathol, 2007. **170**(1): p. 366-76.

88. Song, L., et al., *Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival.* Cancer Res, 2006. **66**(11): p. 5542-8.

89. Li, J., et al., *A chemical and phosphoproteomic characterization of dasatinib action in lung cancer.* Nat Chem Biol, 2010. **6**(4): p. 291-9.

90. Johnson, F.M., et al., *Phase II study of dasatinib in patients with advanced non-small-cell lung cancer.* J Clin Oncol, 2010. **28**(30): p. 4609-15.

91. Haura, E.B., et al., *Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non-small-cell lung cancer.* J Clin Oncol, 2010. **28**(8): p. 1387-94.

92. Heigener, D.F. and M. Reck, *Mutations in the epidermal growth factor receptor gene in non-small cell lung cancer: Impact on treatment beyond gefitinib and erlotinib.* Advances in Therapy, 2011: p. 1-8.

93. Parsons, S.J. and J.T. Parsons, *Src family kinases, key regulators of signal transduction.* Oncogene, 2004. **23**(48): p. 7906-9.

94. Ishizawar, R. and S.J. Parsons, *c-Src and cooperating partners in human cancer.* Cancer Cell, 2004. **6**(3): p. 209-14.

95. Sinibaldi, D., et al., *Induction of p21WAF1/CIP1 and cyclin D1 expression by the Src oncoprotein in mouse fibroblasts: role of activated STAT3 signaling.* Oncogene, 2000. **19**(48): p. 5419-27.

96. Morgan, D.O., et al., *Mitosis-specific phosphorylation of p60c-src by p34cdc2-associated protein kinase.* Cell, 1989. **57**(5): p. 775-86.

97. Yeatman, T.J., *A renaissance for SRC.* Nat Rev Cancer, 2004. **4**(6): p. 470-80.

98. Ellis, L.M., et al., *Down-regulation of vascular endothelial growth factor in a human colon carcinoma cell line transfected with an antisense expression vector specific for c-src.* J Biol Chem, 1998. **273**(2): p. 1052-7.

99. Mukhopadhyay, D., et al., *Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation.* Nature, 1995. **375**(6532): p. 577-81.

100. Laird, A.D., et al., *Src family kinase activity is required for signal tranducer and activator of transcription 3 and focal adhesion kinase phosphorylation and vascular endothelial growth factor signaling in vivo and for anchorage-dependent and -independent growth of human tumor cells.* Mol Cancer Ther, 2003. **2**(5): p. 461-9.

101. Gray, M.J., et al., *HIF-1alpha, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas.* Oncogene, 2005. **24**(19): p. 3110-20.

102. Jiang, B.H., et al., *V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: involvement of HIF-1 in tumor progression.* Cancer Res, 1997. **57**(23): p. 5328-35.

103. Irby, R.B. and T.J. Yeatman, *Role of src expression and activation in human cancer.* Oncogene, 2000. **19**(49): p. 5636-42.

104. Masaki, T., et al., *pp60c-src activation in lung adenocarcinoma.* Eur J Cancer, 2003. **39**(10): p. 1447-55.

105. Song, L., et al., *Activation of Stat3 by receptor tyrosine kinases and cytokines regulates survival in human non-small cell carcinoma cells.* Oncogene, 2003. **22**(27): p. 4150-4165.

106. Wei, L., et al., *Altered regulation of Src upon cell detachment protects human lung adenocarcinoma cells from anoikis.* Oncogene, 2004. **23**(56): p. 9052-61.

107. Johnson, F.M., et al., *Dasatinib (BMS-354825) Tyrosine Kinase Inhibitor Suppresses Invasion and Induces Cell Cycle Arrest and Apoptosis of Head and Neck Squamous Cell Carcinoma and Non-Small Cell Lung Cancer Cells.* Clin Cancer Res, 2005. **11**(19): p. 6924-6932.

108. Yoshida, T., et al., *Effects of Src inhibitors on cell growth and epidermal growth factor receptor and MET signaling in gefitinib-resistant non-small cell lung cancer cells with acquired MET amplification.* Cancer Sci, 2010. **101**(1): p. 167-172.

109. Carretero, J., et al., *Integrative genomic and proteomic analyses identify targets for Lkb1-deficient metastatic lung tumors.* Cancer Cell, 2010. **17**(6): p. 547-59.

110. Johnson, M.L., et al., *Phase II Trial of Dasatinib for Patients with Acquired Resistance to Treatment with the Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors Erlotinib or Gefitinib.* J Thorac Oncol, 2011. **6**(6): p. 1128-31.

111. Herold, C.I., et al., *Phase II Trial of Dasatinib in Patients with Metastatic Breast Cancer Using Real-Time Pharmacodynamic Tissue Biomarkers of Src Inhibition to Escalate Dosing.* Clinical Cancer Research, 2011. **17**(18): p. 6061-6070.

112. Yu, E.Y., et al., *Phase II study of dasatinib in patients with metastatic castration-resistant prostate cancer.* Clinical Cancer Research, 2009. **15**(23): p. 7421.

113. Araujo, J., et al., *Dasatinib and docetaxel combination treatment for patients with castration-resistant progressive prostate cancer: a phase I/II study (CA180086).* J Clin Oncol, 2009. **27**(15 suppl): p. 249s.

114. Yu, E., et al., *A phase II study of once-daily dasatinib for patients with castration-resistant prostate cancer (CA180085).* J Clin Oncol, 2009. **27**: p. 270S-270S.

115. McIntyre, J., J. Castaner, and M. Bayes, *Dasatinib: treatment of leukemia treatment of solid tumors Bcr-Abl and Src kinase inhibitor.* Drugs of the Future, 2006. **31**(4): p. 291-303.

116. Araujo, J. and C. Logothetis, *Dasatinib: a potent SRC inhibitor in clinical development for the treatment of solid tumors.* Cancer treatment reviews, 2010.

117. Kim, L.C., U. Rix, and E.B. Haura, *Dasatinib in solid tumors.* Expert Opinion on Investigational Drugs, 2010. **19**(3): p. 415-425.

118. Luo, F., et al., *Dasatinib (BMS-354825) pharmacokinetics correlate with pSRC pharmacodynamics in phase I studies of patients with cancer (CA180002, CA180003).* Journal of Clinical Oncology, 2006. **24**(18_suppl): p. 3046.

119. Johnson, F.M., et al., *Phase 1 pharmacokinetic and drug-interaction study of dasatinib in patients with advanced solid tumors.* Cancer, 2010. **116**(6): p. 1582-1591.

120. Demetri, G.D., et al., *Phase I dose-escalation and pharmacokinetic study of dasatinib in patients with advanced solid tumors.* Clinical Cancer Research, 2009. **15**(19): p. 6232.

121. Christopher, L.J., et al., *Metabolism and disposition of dasatinib after oral administration to humans.* Drug Metabolism and Disposition, 2008. **36**(7): p. 1357.

122. Wang, X., et al., *Dasatinib pharmacokinetics and exposure-response (ER): relationship to safety and efficacy in patients (pts) with chronic myeloid leukemia (CML).* J Clin Oncol, 2008. **26**: p. 175s.

123. Lilly, M.B., et al., *Dasatinib 140 mg once daily versus 70 mg twice daily in patients with Ph positive acute lymphoblastic leukemia who failed imatinib: Results from a phase 3 study.* American journal of hematology, 2010. **85**(3): p. 164-170.

124. Shah, N.P., et al., *Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and-intolerant chronic-phase chronic myeloid leukemia.* Journal of Clinical Oncology, 2008. **26**(19): p. 3204.

125. Santos, E., et al., *Localization of human prostate cancer through PET imaging using a novel dasatinib analog in mice.* J NUCL MED MEETING ABSTRACTS, 2008. **49**(MeetingAbstracts_1): p. 106P-b-.

126. Kantarjian, H., et al., *Phase 3 study of dasatinib 140 mg once daily versus 70 mg twice daily in patients with chronic myeloid leukemia in accelerated phase resistant or intolerant to imatinib: 15-month median follow-up.* Blood, 2009. **113**(25): p. 6322.

127. Quintás-Cardama, A., et al., *Pleural effusion in patients with chronic myelogenous leukemia treated with dasatinib after imatinib failure.* Journal of Clinical Oncology, 2007. **25**(25): p. 3908.

128. Johnson, M.L., et al., *Phase II Trial of Dasatinib for Patients with Acquired Resistance to Treatment with the Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors Erlotinib or Gefitinib.* Journal of Thoracic Oncology, 2011. **6**(6): p. 1128.

129. Johnson, F.M., et al., *Phase II Study of Dasatinib in Patients With Advanced Non–Small-Cell Lung Cancer.* Journal of Clinical Oncology, 2010. **28**(30): p. 4609.

130. Haura, E.B., et al., *Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non–small-cell lung cancer.* Journal of Clinical Oncology, 2010. **28**(8): p. 1387.

131. Li, D., et al., *BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models.* Oncogene, 2008. **27**(34): p. 4702.

132. Perera, S.A., et al., *HER2YVMA drives rapid development of adenosquamous lung tumors in mice that are sensitive to BIBW2992 and rapamycin combination therapy.* Proceedings of the National Academy of Sciences, 2009. **106**(2): p. 474.

133. Shimamura, T., et al., *Efficacy of BIBW 2992, a potent irreversible inhibitor of EGFR and HER2 in human NSCLC xenografts and in a transgenic mouse lung-cancer model: C7-04.* Journal of Thoracic Oncology, 2007. **2**(8): p. S380.

134. Eskens, F., et al., *A phase I dose escalation study of BIBW 2992, an irreversible dual inhibitor of epidermal growth factor receptor 1 (EGFR) and 2 (HER2) tyrosine kinase in a 2-week on, 2-week off schedule in patients with advanced solid tumours.* British journal of cancer, 2007. **98**(1): p. 80-85.

135. Lewis, N., et al., *A phase I dose escalation study of BIBW 2992, an irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor, in a 3 week on 1 week off schedule in patients with advanced solid tumors.* Journal of Clinical Oncology, 2006. **24**(18_suppl): p. 3091.

136. Yap, T.A., et al., *Phase I trial of the irreversible EGFR and HER2 kinase inhibitor BIBW 2992 in patients with advanced solid tumors.* Journal of Clinical Oncology, 2010. **28**(25): p. 3965.

137. Agus, D., et al., *A phase I dose escalation study of BIBW 2992, an irreversible dual EGFR/HER2 receptor tyrosine kinase inhibitor, in a continuous schedule in patients with advanced solid tumours.* Journal of Clinical Oncology, 2006. **24**(18_suppl): p. 2074.

138. Marshall, J., et al., *A Phase I dose escalation trial of BIBW 2992, an irreversible EGFR/HER2 kinase inhibitor, for 20 and 13 days in combination with docetaxel every 21 days.* Ann. Oncol, 2008. **19**: p. 474.

139. Awada, A., et al., *A phase I dose finding study of the 3-day administration of BIBW 2992, an irreversible dual EGFR/HER-2 inhibitor, in combination with three weekly docetaxel in patients with advanced solid tumors.* J Clin Oncol, 2009. **27**: p. 18.

140. Eschrich, S., et al., *Systems biology modeling of the radiation sensitivity network: a biomarker discovery platform.* Int J Radiat Oncol Biol Phys, 2009. **75**(2): p. 497-505.

141. De Greve, J., et al., *Clinical activity of BIBW 2992, an irreversible inhibitor of EGFR and HER2 in adenocarcinoma of the lung with mutations in the kinase domain of HER2neu.* J Thorac Oncol, 2009. **4**: p. S307.

142. Hirsh, V., *Afatinib (BIBW 2992) development in non-small-cell lung cancer.* Future Oncology, 2011. **7**(7): p. 817-825.

143. Miller, V., V. Hirsh, and J. Cadranel, *Phase IIB/III double-blind randomized trial of afatinib (BIBW 2992, an irreversible inhibitor of EGFR/HER1 and HER2) plus best supportive care (BSC) versus placebo plus BSC in patients with NSCLC failing 1–2 lines of chemotherapy and erlotinib or gefitinib (LUX-Lung 1)[abstract LBA1].* Ann Oncol, 2010. **21**.

144. Miller, V., V. Hirsch, and J. Cadranel. *Subgroup analysis of LUX-Lung 1: a randomized Phase III trial of afatinib (BIBW 2992)+ best supportive care (BSC) versus placebo+ BSC in patients with NSCLC failing 1–2 lines of chemotherapy and erlotinib or gefitinib*. 2010.

145. Yamamoto, N., T. Tamura, and T. Takahashi, *Phase I openlabel trial of continuous dose of BIBW 2992 in patients with advanced non-small cell lung cancer failing chemotherapy and/or erlotinib and/or gefitinib (LUX-Lung 4)[abstract 230].* J Thorac Oncol, 2010. **5**: p. 91.

146. Yang, C., et al., *A Phase II study of BIBW 2992 in patients with adenocarcinoma of the lung and activating EGFR mutations (LUX-Lung 2).* J Clin Oncol, 2010. **28**: p. S7521.

147. Stopfer, P., et al., *Pharmacokinetics (PK) of [14C]-BIBW 2992 after administration of a single dose of 15 mg [14C]-BIBW 2992 oral solution in healthy male volunteers.* J Clin Oncol, 2008. **26**: p. 15.

148. Drew, B.J., et al., *Prevention of Torsade de Pointes in hospital settings: a scientific statement from the American Heart Association and the American College of Cardiology Foundation endorsed by the American Association of Critical-Care Nurses and the International Society for Computerized Electrocardiology.* Journal of the American College of Cardiology, 2010. **55**(9): p. 934.

149. Song, L., Morris, M., Bagui, T., Lee, F., Jove, R., and Haura, E.B., *Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival.* Cancer Res, 2006. **In Press**.

150. Wacker, B., et al., *Correlation between development of rash and efficacy in patients treated with the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib in two large phase III studies.* Clinical cancer research, 2007. **13**(13): p. 3913.

151. Drew, B.J., et al., *Prevention of Torsade de Pointes in Hospital Settings.* Circulation, 2010. **121**(8): p. 1047-1060.

152. Smith, A.J., *Medication-Induced QT-Interval Prolongation and Torsades de Pointes.* US Pharm, 2011. **2**: p. 18.

153. Chen, Y., et al., *P-glycoprotein and breast cancer resistance protein influence brain distribution of dasatinib.* Journal of Pharmacology and Experimental Therapeutics, 2009. **330**(3): p. 956.

154. Balagula, Y., M.E. Lacouture, and J.A. Cotliar, *Dermatologic toxicities of targeted anticancer therapies.* J Support Oncol, 2010. **8**: p. 149-161.

155. Miller Jr, R.G., *What Price Kaplan-Meier?* Biometrics, 1983: p. 1077-1081.

156. Meier, P., et al., *The Price of Kaplan-Meier.* Journal of the American Statistical Association, 2004. **99**(467): p. 890-896.

[1] SPRYCEL® (dasatinib) Tablets Prescribing Information. Bristol-Myers Squibb Company, Princeton, NJ. June 2006.

[2] SPRYCEL® (dasatinib) BMS-354825, Bristol-Myers Squibb Investigator Brochure, Version #5, 2006.

[3] SPRYCEL® (dasatinib) BMS-354825, Bristol-Myers Squibb Investigator Brochure, Version 8, 2008

TITLE PAGE

Phase I Trial Evaluating Safety and Tolerability of the Irreversible Epidermal Growth Factor Receptor Inhibitor Afatinib (BIBW 2992) in Combination with the SRC Kinase Inhibitor Dasatinib for Patients with Non-small Cell Lung Cancer (NSCLC)

BI Protocol Number: 1200.166
BMS Protocol Number: CA180-379
MCC Protocol Number: MCC 17176
~~Liberty~~ Chesapeake IRB Protocol Number: Pro0001453112.09.0006

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Version ~~23~~.0 ~~December 27~~March 22, ~~6~~2017

~~**Initial Moffitt SRC approval date (v1.6): August 7, 2012**~~
~~**Initial Liberty IRB approval date (v1.6): September 10, 2012**~~
~~**Moffitt SRC approval date (v1.7): September 16, 2013**~~
~~**Liberty IRB approval date (v1.7): September 26, 2013**~~
~~**Moffitt SRC approval date (v1.8): June 30, 2014**~~
~~**Liberty IRB approval date (v1.8): July 9, 2014**~~

7 OBSERVATIONS

7.1 Methodology

7.1.1 Plasma Collection and Processing

Blood samples are to be collected from an indwelling catheter or by direct venipuncture. If a catheter is used for blood collection, then approximately 10 mL of blood should be withdrawn initially and discarded.

7.2 Correlative Science

7.2.1 Stored Plasma Samples ~~- VEGF~~

Previous studies have indicated that SRC inhibitors can reduce tumor production of p-SRC, VEGF and IL-8 that are important factors for tumor angiogenesis. Blood will be collected at the time of screening or day 1, and again on day 29 to assess for future correlative markers with a purple-top EDTA tube. These samples will be processed and analyzed for plasma-based molecular markers. ~~Any study on these blood samples would be performed retrospectively under a separate protocol(s) or amendment, with separate IRB approval.~~ Serum/Plasma will be centrifuged for 1 minute at 12,000 rpm to pellet any floating debris/cells, and then transferred to a fresh microcentrifuge tube and immediately frozen and stored at –80°C. ~~These blood samples will not be used for any studies without a specific study protocol which has an explicit IRB approval for that purpose.~~

These samples will be processed and analyzed for plasma-based molecular markers. Specifically, plasma is extracted and assessed for epidermal growth factor receptor (EGFR) mutation type and copy number. This testing is done using digital droplet PCR with specifics primers for EGFR L858R, Exon 19 del, and T790M. This testing is performed by collaborator (Biodesix), using an established and executed material transfer agreement (Biodesix-Moffitt 113016). Coded plasma aliquot samples are shipped to collaborator by Moffitt Tissue Core, and coded .csv file containing the number of relevant reads per pooled aliquot is then sent back to the investigator by the collaborator.

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Summary of Changes – MCC17176

V2.0

Section	Description of Changes	Rationale
Title page; Table of Contents	Updated to version 2.0; added information on v1.7 and v1.8 approvals; formatting changes; co-investigator list removed; CTC removed; page numbers revised	Updated information applicable to amendment. Removed staff lists as they are not needed and have changed throughout study. Page numbers revised according to revisions.
Protocol Synopsis	Afatinib lowercase	Formatting change
Protocol Synopsis	Inclusion and Exclusion Criteria deleted	Inclusion and Exclusion Criteria deleted in order to eliminate inconsistency with main section.
Protocol Synopsis	Deleted “An elective patient decline questionnaire will be included after screening to guide corrective action.”	Questionnaire was never developed.
Study Calendar – Phase IA	Afatinib uppercase	Formatting change
1.1.6 Significance of the Src-TKI Dasatinib in NSCLC	SRC capitalized	Formatting change
Table 1: Drugs that may overcome EGFR resistance	Recept deleted	Formatting change
4.1 Overview	Name of IRB deleted	This information is subject to change and was removed.
5.2 Exclusion Criteria	Criteria #3 now specifies that only women of childbearing potential must have a negative pregnancy test.	Clarify that women who are not of child bearing potential should not be subjected to a pregnancy test.
5.2 Exclusion Criteria	Criteria #17 edited to explain that trace pericardial or pleural effusion is acceptable among subjects at baseline.	Clarify that not all pericardial and pleural effusion not allowed on study.
5.2 Exclusion Criteria	Criteria #19 added azithromycin to the list of P-gp inhibitors that are not allowed.	Modified per guidance on P-gp inhibitors.
5.2 Exclusion Criteria	ANC <1000 is exclusion cut off instead of 1500.	Revised per letter to IRB that was previously approved.
6.1 Study Calendar	Pregnancy test for women of child-bearing potential.	Edited to conform with wording used in Exclusion criteria.

6.2.1 Screening Evaluation, 6.3.2 Definition of DLT; 7.3 Assessment of Safety	NCI-CTCAE spelling	Typographical error
6.2.2 Cycle 1	Dosing for Phase 1B explained.	Revised for consistency with other sections
6.2.2 Cycle 1	C1D15 description	This applies to both Phase 1A and Phase 1B. Requirement to take study drug in clinic removed.
6.4.1.1 Prohibited Therapies	Carvedilol and quinidine removed from prohibited P-Glycoprotein	List revised
6.6 Patient Withdrawl Criteria	Remove description of subject visit.	All subjects already assessed for new related 21 calendar days; however 30 day patient visit is not feasible so removed.
6.11 Management of Adverse Events	SPF changed; number of weeks of minocycline treatment;	

Cover Letter
Submission Type: ICF Update
Liberty IRB, Inc
1450 S Woodland Blvd DeLand, FL 32720

June 9, 2014

Re: Liberty trial protocol 12.09.0006

Dear Staff of Liberty Institutional Review Board,

I am writing to notify your office of an amendment to protocol for Liberty 12.09.0006, (BI 1200.166, MCC 17176), "Phase I Trial Evaluating Safety and Tolerability of the Irreversible Epidermal Growth Factor Receptor Inhibitor Afatinib (BIBW 2992) in Combination with the SRC Kinase Inhibitor Dasatinib for Patients with Non-small Cell Lung Cancer (NSCLC)".

We have received helpful commentary from the Boehringer-Ingelheim office. On the basis of this feedback, we have further refined the clinical trial protocol. Included is the trial protocol word document with tracked changes. These changes primarily focused on refining the DLT definition and clarifying discrepancies present in the prior version.

Enclosed in this submission is an updated protocol with tracked changes.

Also attached is the summary of changes on pages 2 and 3.

Thank you for your time and consideration.

Sincerely,

Ben Creelan

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Summary of changes:

1. Changed eligibility so that erlotinib may be continued until up to 3 days of C1D1. The mean terminal half-life of erlotinib (OSI-774) is 7 hours in healthy subjects, and closer to 24 hours at steady-state in cancer subjects. The overall rationale for this amendment is to avoid flare phenomenon from erlotinib withdrawal.
2. Changed dasatinib tablet specification to reflect actual dasatinib tablets received (50 mg and 20 mg tablets)
3. Included the 20 mg afatinib tablet supply received in Product Specifications section
4. Clarified imaging schedule as first evaluation at day 50, and then every 8 weeks thereafter.
5. Removed mandatory pelvis CT from imaging schedule (this was SOC and does not affect budget)
6. Made screening period tests uniformly within 28 days prior to C1D1. (some areas had said 30 days).
7. Specified purple-top tube for plasma samples On page 10 and page 53
8. Inclusion Criteria #5, and Core guidance for Afatinib ALT & AST $\leq$ 2.5 ULN, not $\leq$ 3 ULN as in exclusion criteria. The exclusion criteria has been amended to $\leq$ 2.5.
9. Removed inclusion criteria #5, since it redundant with exclusion criteria #23
10. Specified that afatinib should be taken 1 hour before/ 2 hours after meals.
11. Specified a flexibility of +/- 3 days for C1D8 and C1D15.
12. On page 12 and page 55, Exclusion Criteria # 19, and Additional pgp inhibitor drugs in the BI core protocol have been added to this exclusion criterion, and also page 61, under Section 6.4.1.1.
13. On pages 31, 32 & 33, Section 2.3.1 Table 2 has footnotes have been defined.
14. On page 50, Section 3.5, add “light” to blue color.
15. On page 52, Section 4.1, value of 50 days changed to 56 days to align with timeframe in study schema.
16. On page 60, under Section 6.3.2, for clarification: Page 67 of protocol stated “In the event of CTCAE Grade $\geq$ 3 rash, treatment with afatinib and dasatinib should be paused until recovery to CTCAE Grade $\leq$ 1. Treatment should be resumed at a reduced dose per Appendix, “Papulopustular Skin Rash”.”
    Pursuant to this, 6.11.1 section was revised to distinguish between routine CTCAE grade 3 skin rash, and “severe” skin rash. This definition of severity and management algorithm is based upon expert opinion. Lacouture et al, Expert Rev. Anticancer Ther 13(6) 2013.
    On the basis of above, CTC grade 2 and routine grade 3 skin rash will be managed identically, with a separate management plan for “severe grade 3 skin rash”. Only severe grade 3 skin rash will be considered a DLT, as the amendment language now states. See tracked changes.
    Along these lines, Section 6.3.2 has been amended to include “severe grade 3 rash refractory to optimal supportive care” under DLT definition.

17. On page 87, under Section 8.1.2, added "light" to blue color.
18. Amended Section 9.3 to reflect voluntary reporting to MedWatch under GCP only for unexpected drug-related SAEs. Note the reporting responsibilities to Boehringer using BI CRF forms remain unchanged.
19. Removed Section 9.4, 9.5. Based upon feedback from our regulatory specialist, this section was redundant and non-applicable, because this particular trial is IND exempt. The AE reporting plan and guidelines are already outlined in Section 9.3.
20. Amended Section 9.1.1: Adverse events section. Based upon feedback from our internal monitors, clarified reporting plan for adverse events to specify: "Select events, regardless of grade, will be recorded as an AE: diarrhea, skin rash, thrombocytopenia, neutropenia, and AST/ALT, bilirubin or creatinine elevation. Other changes in vital signs, ECG, physical examination and laboratory test results will not be recorded as an AE in the CRF, unless there are clinical signs or symptoms, or they required clinical intervention or treatment."
21. Amended Section 6.1, 6.2.1: Amended screening procedure window to be consistent and uniform 28 days prior to C1D1, instead of 30 days.

Re: MCC 17176

September 9, 2013

Summary of changes for SRC

- Title Page: updated Principal investigator based upon mutual agreement with Dr Haura and Dr Creelan.
- Title Page: updated clinical trial coordinator and contact information
- Title Page, and onwards: Updated relevant nomenclature throughout protocol, from investigational drug name (BIBW 2992) to current generic drug name (afatinib).
- Page 44 : updated clinical experience based on most recent investigator's brochure from Jan 15 2013, including the FDA approval of afatinib on July 12, 2013.
- Page 46: updated information regarding the clinical trial experience leading to FDA approval of afatinib for advanced NSCLC
- Page 49: updated Table 7 to reflect status of ongoing trials
- Page 120: Included two new appendices, Appendix 2 and Appendix 3. These independent appendices were stipulated to be included in the trial protocol based upon contractual agreement with the sponsor, Boehringer-Ingelheim.

Sincerely,

Ben Creelan, MD