FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE

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Title of Protocol:
ImmunoRad: Stratified Phase II Trial of Image Guided Hypofractionated Radiotherapy with concurrent Nelfinavir and Immunotherapy in Advanced Melanoma, Lung Cancer, and Renal Cell Carcinoma

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Emergency number (24 hours) for Seattle Cancer Care Alliance/University of Washington Medical Center patients: 206-598-6190.

PROTOCOL SYNOPSIS

Protocol Title	IMMUNORAD: A STRATIFIED PHASE II TRIAL OF HYPOFRACTIONATED RADIOTHERAPY WITH NELFINAVIR AND IMMUNOTHERAPY IN ADVANCED MELANOMA, NSCLC, AND RCC
Short Title	IMMUNORAD
Protocol Number	CCIRB #9712
Protocol Sponsor	None
Trial Phase	Phase II
Methodology	Open Label
Study Center	Single Center
Study Objectives	1. Primary objective is to estimate the clinical response as quantified by irRECIST 1.1 to hypofractionated radiotherapy with nelfinavir and anti-PD1/PDL1 immunotherapy. 2. Secondary objectives are to evaluate late toxicity, immune-related clinical responses and immune pharmacodynamic changes hypofractionated radiotherapy with nelfinavir and anti PD1/PDL1 immunotherapy
Diagnosis and Main Inclusion Criteria	Patients with previously untreated or previously treated metastatic melanoma, NSCLC, or renal cell carcinoma with bone, lung, liver, or subcutaneous or nodal involvement but without evidence of brain involvement. Patients who have previously been treated with radiation will be included in the study, so long as a suitable plan for treatment can be developed. Patients who have received prior immunotherapy will be included in the study and enrolled into a separate stratum.
Study product, dose, route, regimen	Hypofractionated Image Guided Radiation Therapy. Three fractions of 800cGy each given over 3-14 days. Pembrolizumab, Nivolumab, or Atezolizumab per standard dosing and schedule. Nelfinavir: 1250 mg PO BID administered for 7 – 14 days prior to start of immunotherapy, for a total of 11-12 weeks.
Duration of administration	Radiation treatment to be delivered over 3-14 days with concurrent Nelfinavir and Immunotherapy. Subjects will take nelfinavir for a total of 11-12 weeks. Immunotherapy will continue per the FDA-approved dosing schedule.
Reference Therapy	FDA-approved PD-1 or PD-L1 inhibitors
Number of trial subjects	The expected accrual rate = 30 pts/yr. The protocol is designed for a maximum total of 120 patients (10 patients per year for melanoma and 10 patients per year for NSCLC and 10 patients per year for RCC).
Study Duration	The expected total study duration is 4 to 4 1/2 years needed to complete accrual.
Statistical Methodology	This is a stratified phase II study of hypofractionated radiotherapy combined with immunotherapy for previously untreated or previously treated metastatic melanoma, NSCLC, and renal cell carcinoma patients. Phase II is stratified by histology and whether or not the patient has received prior immunotherapy. We will also collect serum for correlative studies.

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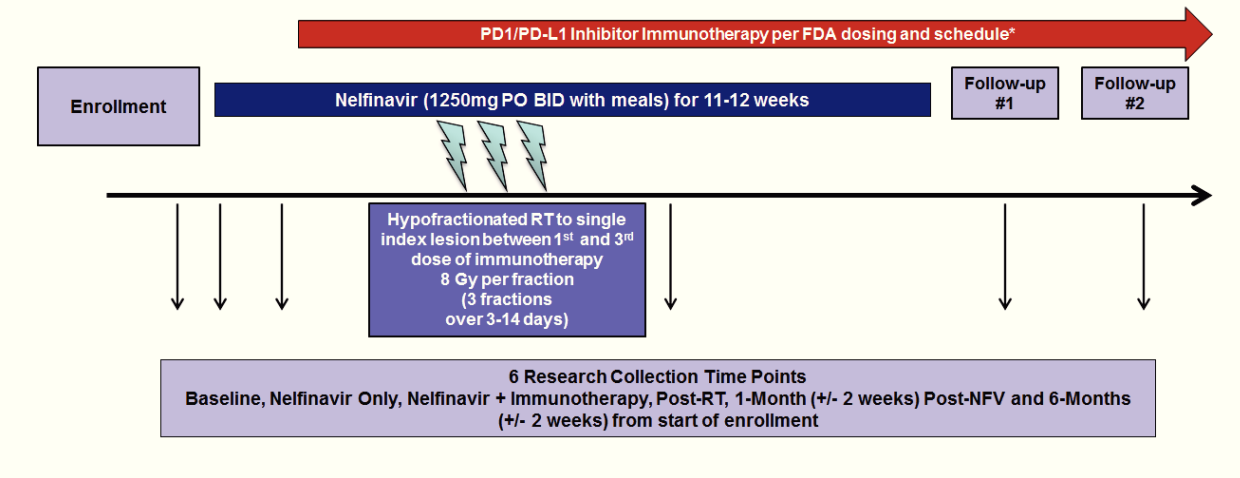


	Baseline	Nelfinavir Only	Nelfinavir + 1st Dose Immunotherapy	Hypofractionated Radiotherapy	Post Hypofractionated Radiotherapy	Final Dose of Nelfinavir	Follow-up #1	Follow-up #2
				Immunotherapy every 2-3 weeks until progression				
		11-12 weeks of Nelfinavir					1-Month Post NFV	6-Months from Enrollment
Tests and Observations							(+/-) 2 Weeks	(+/-) 2 Weeks
Physical Exam; ECOG	x		x	x	x	x	x	x
Adverse Events[g]		x	x	x	x	x	x	x
Drug Diary[g]		x	x	x	x	x		
CT Scan[a]	x						x	x
irRECIST 1.1[a, g]	x						x	x
MRI Brain[b]	x							
Biopsy[c, g]							x	x
Laboratory								
CBC w/diff, Hgb, Platelets[d]	x	x	x		x			
CMP[d]	x							
Pregnancy Test[e]	x							
Research Blood Collection[g,h]	x	x	x		x		x	x
Treatment								
Nelfinavir[g]		x	x	x	x	x		
Immunotherapy[i]			x	x	x	x	x[i]	x[i]
3 days x 6-8 Gy[f]				x				

KEY

a. Baseline within 2 months of study entry. Repeat every 10-12 weeks or as clinically indicated
b. Within 2 months of study entry
c. Optional biopsy at end of service
d. Within 30 days of study entry
e. For WOCBP; Within 7 days of study entry
f. A CT simulation will be scheduled to plan for 3 fractions of 6-8gy each to a single lesion. Radiation will commence between 1st and 3rd dose of immunotherapy.
g. Research Procedure
h. 8 yellow top BD #364606 + 1 red top # BD 367820 (Louie King specimen processing)
i. Pembrolizumab, Nivolumab, or Atezolizmab. Continue until progression.

1.0 INTRODUCTION TO THE PROTOCOL

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guideline), applicable government regulations and Institutional research policies and procedures.

1.1 Introduction

Therapies targeting immune check points represent one of the most exciting breakthroughs against lung cancer, notwithstanding the targeted therapies against known driver mutations. These therapies that target immune check points have the capability to dramatically change the field and provide a more physiological way of harnessing innate immune responses against the patient's tumors. Several studies have been published/ongoing studying various aspects of PD1/PD-L1 or CTLA4 and they are summarized in recent reviews (1)

Non-small cell lung cancers (NSCLCs) are among the solid tumors with the highest frequency of somatic mutations (8.17 and 6.43 mutations per Mb (Megabase) for the squamous and non-squamous subtype, respectively), only surpassed by melanoma at 13.2 mutations per Mb (2). These mutations, likely secondary to DNA damage from cigarette smoke and UV exposure, have the potential to generate tumor-rejection antigens. However, the vast majority of lung cancers successfully evade elimination by the immune system. This is in part attributable to the expression by cells in the tumor microenvironment of molecules such as PD-L1 that engage cognate inhibitory receptors (PD-1) expressed on T cells. PD-L1 is expressed by cells in many lung cancers, either constitutively or in response to cytokines produced by tumor-infiltrating T cells. Signaling through the PD-1/PD-L1 axis can inhibit cytokine production and cytolytic activity of CD8+ and CD4+ T cells that have migrated into the tumor. The last decade has witnessed the development of several novel agents that block these immune "checkpoints" (3) Inhibiting PD-L1 and dis-engaging PD-1 on T cells in cancer patients can reverse the adaptive immune resistance, and enhance T cell-mediated anti-tumor immunity (4)

Early studies with anti-PD-L1 antibodies in patients with a variety of tumor types have shown response rates of 25-34%, with some durable responses (5). Interestingly, tumors with the greatest mutational load and heterogeneity, such as NSCLC, melanoma, and renal cell carcinoma, demonstrated the greatest benefit. Recent data suggest that both tumor heterogeneity and PD-L1 expression are associated with higher response rates to PD-L1-directed therapies (5). Aside from tumor PD-L1 expression, success of the anti-PD-L1 therapy relates to the presence of pre-existing immunity that is suppressed by PD-1/PD-L1 signaling and becomes re-invigorated following therapy with PD-L1 blockade(6). Herbst et al. demonstrated a systemic re-priming and expansion of both pre-existing antitumor T-cell and non-tumor-directed T-cell populations in the peripheral blood (5). Other studies in melanoma and prostate cancer patients have revealed that specific host T cell repertoire, determined by next-generation sequencing (of T cell receptor, TCR sequence usage) have revealed that a less diverse, more clonal population accurately predicted responders from non-responders to immune check-point blockade (7).

Preclinical data demonstrates that radiation can improve responses of lung cancer to PD-L1 immunotherapy (4). The mechanisms of cooperation and improved disease control have not been clearly established but may include: (1) enhanced immunogenic antigen presentation (2) stimulation of cytokine release; and (3) enhanced PD-L1 expression on the tumors resulting from change in the stromal lymphocyte infiltration induced by XRT. Blockade of PD-L1 near the time of radiotherapy may enhance the native lymphocyte response. The role of hypofractionated or SBRT/HIGRT-induced tumor antigen release in the era of combination checkpoint inhibition: Higher doses of XRT delivered over shorter fractions have the ability to increase antigen presenting cells (8). Wolchok et al. recently reported a 40% response rate to combination therapy, with all patients achieving greater than 80% or more reduction in tumor burden in advanced melanoma. In our proposed model, HIGRT/SBRT is complementary to immune checkpoint inhibition and would be anticipated to augment combination therapy.

1.2 Preclinical Data

Nelfinavir and radiosensitization:

We and others have been attempting to identify a common downstream signal that is associated with radiation resistance. Data from our laboratory have demonstrated that inhibition of Ras and/or the downstream PI3K-Akt pathway increases the radiosensitivity of cells in which this pathway is activated but does not affect cells without activation of this pathway (including normal tissues)(9, 10). We have both preclinical and clinical experience with farnesyltransferase inhibitors as radiation sensitizers (11, 12). However, inhibition of targets downstream of Ras including PI3K may provide a more effective target as this pathway is affected by both mutations and/or overexpression of EGFR, PTEN, Ras, and others. There are, however, currently no clinically useful inhibitors of PI3K. PI3K phosphorylates PtdIns-4, 5-P2 to yield PtdIns-3, 4, 5-P3. PtdIns-3, 4, 5-P3 in turn causes membrane localization of protein kinase B (PKB/Akt) and the phosphoinositide-dependent kinases (PDK's) which phosphorylate Akt (13). Akt thus is an immediate downstream target of PI3K.

It has been reported that the activation (phosphorylation) of Akt by insulin is reduced in the presence of the HIV protease inhibitor (HPI) NFV (nelfinavir) (14). HPIs have also been reported to cause insulin resistance and diabetes(15). We know that Akt signaling plays a role in insulin signaling so we speculated that these side-effects of HPIs might be due to interference with Akt signaling. Our hypothesis is that HPI's will inhibit Akt signaling and radiosensitize human tumors as a result. Akt is a serine/threonine kinase that is phosphorylated at two sites, Thr 308 (kinase domain) and Ser 473 (C-terminal regulatory region). It is the Ser 473 site that appears to be necessary for maximal activation of Akt(16). We tested the human head and neck cancer cell line SQ20B with a constitutively active EGFR receptor and the human bladder carcinoma cell line T24 with a v12 mutation in H-Ras (Figure 1). At a clinically relevant dose of 5 μM, NFV down regulated Akt phosphorylation at Ser 473. There was no effect on Akt phosphorylation at Thr 308. Total Akt levels remained constant. Increasing the concentration of NFV slightly increased the onset of the response, but resulted in cell toxicity at 20 μM. Concentrations in the 5-10 μM range had no effect on cell growth rate of either T24 or SQ20B cells (data not shown).

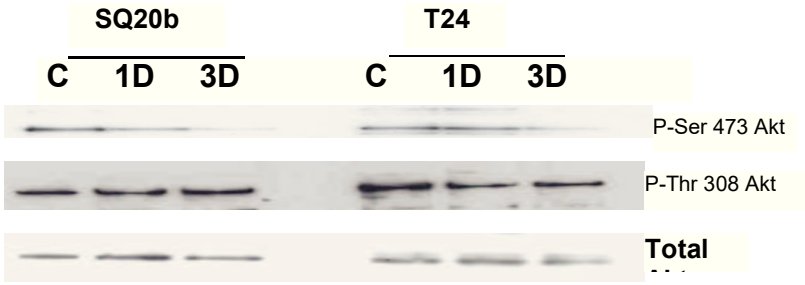


Figure 1: SQ20B or T24 cells were treated with 5 μM NFV for 1 or 3 days. Cells were harvested and immunoblotted with antibody used to detect the active or phosphorylated forms of Ser 473 and Thr 308 Akt. Antibody detecting total Akt was also used.

After showing that NFV downregulates P-Akt, we then evaluated radiosensitization in several human tumor cell lines that have EGFR-Ras-PI3K-Akt pathway activation (SQ20B, T24, MIAPACA2, and A549). Rat embryo fibroblasts (REF) were evaluated to assess radiosensitization in cell lines without this pathway activation. Table 1 displays the data from the clonogenic survival curves in tabular form. In every cell line with increased signaling through Akt, there was at least a 19% reduction in surviving fraction with NFV and in many cases the reduction was as large as 40%. There was no change in the radiosensitization of REF cells. Patients treated with radiation generally receive 30 + a treatment of 1.8-2 Gy and the difference is thus exponentially driven. For example, with an SF2 of 0.56 and 30 fractions of 2 Gy, the survival would be 0.56 EE 30 = 2.8 X 10^{-8}. If the SF2 is 0.45 and 30 fractions of 2 Gy, then the survival would be = 3.95 X 10^{-11}. That translates to almost a 3 log difference in the cell kill.

Table 1: Surviving fraction after 2 Gy with and without 5 μM NFV.

Cell Line	Cancer Type	Mutation	Control SF2 (mean +/- SD)	Nelfinavir SF2 (mean +/- SD)
T24	Bladder	H-Ras	n=6 0.560 +/- 0.061	n=6 0.452 +/- 0.023 p=0.006*
SQ20B	H&N	EGFR	n=6 0.691 +/- 0.042	n=5 0.401 +/- 0.024 p<0.001*
MIAPACA2	Pancreatic	K-Ras	n=4 0.906 +/- 0.086	n=5 0.526 +/- 0.040 p=0.001*
A549	Lung	K-Ras	n=6 0.570 +/- 0.042	n=4 0.401 +/- 0.062 p<0.001*
REF	Rat Fibroblasts	None	n=5 0.408 +/- 0.040	n=5 0.397 +/- 0.054 p=0.89*

* **compared to Control SF2 by t-test**

Since the effectiveness of cell kill by cis-platinum and etoposide can also be modified with down-regulation of Akt, we tested for sensitization with nelfinavir. T24, SQ20B, and A549 cells were treated with 100, 10, or 1 μM cis-platinum or etoposide with and without 10 μM NFV. Sensitization to cis-platinum or etoposide with the addition of NFV was not seen in any of the cell lines. We basically saw one log of cell kill at 1 μM cis-platinum and 10 μM of etoposide which was unchanged with the addition of NFV.

In vivo studies were also used to assess radiation sensitization. Mice bearing SQ20B tumors were randomly assigned to each treatment arm (radiation plus drug, radiation alone, drug alone, or mock treatment). Mice were pre-treated for 5 days with oral NFV. The serum concentration of nelfinavir achieved was in the 2-5 μM range. Figure 2 shows the *in vivo* down regulation of Akt in SQ20B xenografts with NFV. **A** shows immunohistochemistry (400X) of representative SQ20B tumor from a control mouse (left) and a mouse treated with NFV (right). The tumors were harvested 5 days after being treated with placebo or NFV 0.6 mg/day. **B** shows an immunoblot of the lysate from the tumors shown in A.

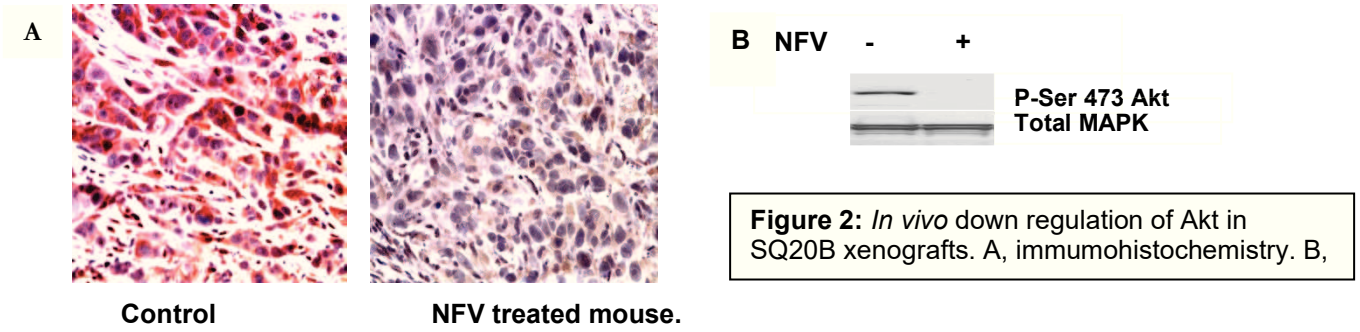
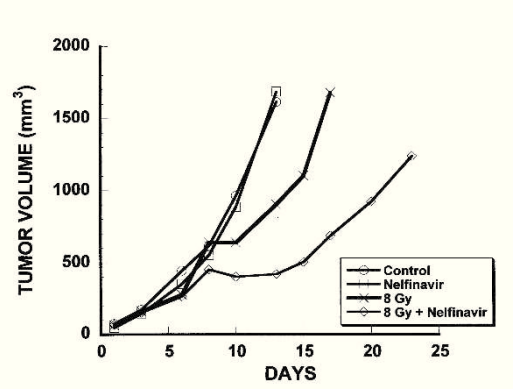


Figure 2: *In vivo* down regulation of Akt in SQ20B xenografts. A, immumohistochemistry. B,

For tumor re-growth, 8 Gy was chosen because this dose leads to a growth delay and not a cure thereby permitting the detection for statistical synergy between radiation and drug. The figure on next page shows the data on SQ20B xenografts treated with NFV. The mean tumor volumes are shown in Figure 3A. In the radiation plus NFV group, two slowly growing tumors reached a volume of 1000 mm^3 at 70 and 78 days. The mean time to tumor volume of 1000 mm^3 (Figure 3B) was 11 days in the control group and 12 days in the NFV alone group. As expected, mean values increased in both radiation alone (15 days) and radiation and NFV (41 days) groups. By linear regression analysis, a statistically significant synergistic effect between radiation and NFV was detected (p=0.03).

A



B

Days to tumor volume of 1000 mm^3 Mean ± SD (range)			
Control	N	8 Gy	8 Gy + N
$n = 6$	$n = 6$	$n = 5$	$n = 5$
11.0 ± 1.5	12.0 ± 1.5	15.0 ± 2.0	41.0 ± 30.3
(10 – 13)	(10 – 13)	(13 – 17)	(17 - 78)
Test of synergy (one-sided)		$P = 0.03$	

Figure 3: Re-growth delay of SQ20B xenografts +/- NFV after radiation. **A** shows the mean tumor volume in control tumors (○), NFV treated tumors (□), tumors treated with 8 Gy radiation (x), or 8 Gy and NFV treated tumors (◊). **B** is a table that shows mean days to reach tumor volume 1000 mm^3 for the 4 treatment groups and the significance of the test of synergy between radiation and NFV.

We evaluated normal tissue toxicity *in vivo* in mice after administration of NFV and radiation. The right leg of each mouse was irradiated with 8 Gy (2 control mice and 2 treated with NFV). We assessed the mice weekly for the visual development of skin fibrosis and leg contractures. No differences were observed in normal tissue toxicity between the 4 groups of extremities (control unirradiated, control irradiated, NFV unirradiated, NFV irradiated). At 60 days, the mice were sacrificed and histological sections of legs were compared. There was an increase in epidermal thickness with irradiation (mean control 16 μM vs. 22 μM for 8 Gy). It was however impossible to tell which mouse had been given the NFV vs. control and no additive effect on skin fibrosis was seen with NFV and radiation.

Pre-clinical evidence of immune modulation with NFV and radiation
The tumor regrowth assays with xenografts in nude mice. The combination of nelfinavir and radiation increased time to regrowth compared with radiation alone whereas nelfinavir alone had little effect on tumor regrowth. This radiosensitizing effect was significantly greater than suggested by in vitro clonogenic survival assays. One possible explanation for the discordance is that nelfinavir has an immunomodulatory effect in combination with radiation. This is an augmentation of response that would only be evident *in vivo* and not in *in vitro* assays.

Immune modulation with radiation and immune checkpoint (PD-1) inhibition: Preclinical data demonstrate that radiation can improve responses of lung cancer to PD-L1 immunotherapy(4). The mechanisms of cooperation and improved disease control have not been clearly established but may include: (1) enhanced immunogenic antigen presentation (2) stimulation of cytokine release; and (3) enhanced PD-L1 expression on the tumors resulting from change in the stromal lymphocyte infiltration induced by XRT. Blockade of PD-L1 near the time of radiotherapy may enhance the native lymphocyte response. The role of hypofractionated or SBRT-induced tumor antigen release in the era of combination checkpoint inhibition: Higher doses of XRT delivered over shorter fractions have the ability to increase antigen presenting cells (8). Wolchok et al. recently reported a 40% response rate to combination therapy, with all patients achieving greater than 80% or more reduction in tumor burden in advanced melanoma. More recently, blockage of PD-L1 has been demonstrated to be superior to everolimus in advanced renal cell carcinoma with an overall response rate of 25%. This led to the FDA approval of nivolumab as second line therapy in these patients. FDA has rapidly given approval to additional drugs that belong to the same class as nivolumab for its identical clinical indications in melanoma, non-small cell lung cancer, and renal cell carcinoma. In our proposed model, HIGRT/SBRT is complementary to immune checkpoint inhibition and would be anticipated to augment combination therapy.

Immune modulation with dual P-I-3 Kinase and Immune Checkpoint (PD-1) Inhibition:
Kim et al investigated epigenetic modulation as a strategy to augment clinical response to immune checkpoint inhibition in a pre-clinical model of CT26 tumors or metastatic 4T1tumors. Co-treatment with epigenetic-modulating drugs and checkpoint inhibitors markedly improved treatment outcomes, curing more than 80% of the tumor-bearing mice. Functional studies revealed that the primary targets of the

epigenetic modulators were myeloid-derived suppressor cells (MDSCs). Furthermore, they observed that the underlying mechanism driving this improved response was PI3K inhibition and that inhibition of this enzyme reduced circulating MDSCs also eradicated 4T1tumors in 80% of the mice when combined with immune checkpoint inhibitors.(17) This provides strong pre-clinical supportive data that P-I-3 kinase inhibition can augment response to immune checkpoint blockade.

1.3 Clinical Data to Date

Hypofractionated Image Guided Radiotherapy (HIGRT) or Stereotactic Body Radiation Therapy (SBRT)
Hypofractionated image guided radiotherapy or Stereotactic Body Radiation Therapy (HIGRT/SBRT) is a highly precise treatment technique that delivers large tumoricidal doses of radiation to a small tumor. In this protocol, we will use these terms interchangeably. Although this represents one of the most exciting and active frontiers of research in the radiotherapeutics' management of early stage NSCLC, this treatment technique was originally developed in 1951 by a Swedish neurosurgeon, Lars Leksell for the treatment of intracranial metastases.(18) Hypofractionation allows for escalation of dose without extending the overall treatment duration, as would be the case with conventional fractionated radiotherapy. A Phase I dose-escalation trial evaluated patients with T1-2 N0 NSCLC with no restriction on tumor location. Each treatment course was administered over 3 fractions with a starting dose of 8 Gy per fraction. Patients were stratified into 3 dose-escalation groups based on T stage and size (T1, T2 <5 cm, and T2 5–7 cm). This trial demonstrated that the maximally tolerated dose for T2 tumors larger than 5 cm was 22 Gy × 3 for, and was not reached at 20 Gy × 3 for T1 tumors or at 22 Gy × 3 for T2 tumors smaller than 5 cm.(19). There were a total of 10 local failures in the 47 patients treated in this study with nine local failures in patients treated to the lower dose levels (<16Gy x 3). A Phase II trial from the same group of investigators treated 70 patients with Stage I NSCLC with the doses established in the phase I study. With a median follow-up of 17.5 months, the local control was 95%, which appears at least as effective as a definitive surgical resection. Severe toxicity occurred at a median of 10.5 months in 17% of those patients with peripheral lesions versus 46% with central lesions.(20) Several other institutions have subsequently published their experience utilizing SBRT/HIGRT for early lung cancer with a variety of dose fractionation and prescription schemes. The initial data appear promising with 80%–100% local control, 40%–100% 2- to 3-year survival, and 0%–4% grade 3 toxicity), although in general the median follow-up for these studies is relatively short.(21-27). Given these promising results, the RTOG (RTOG 0618) has initiated a phase II study of stereotactic body radiotherapy for operable patients with early stage operable NSCLC. Additionally, Dr. Robert Timmerman initiated a randomized trial of surgical resection vs. stereotactic body radiation for early stage lung cancer in early 2010 (RTOG 1021). While the results of these studies are eagerly anticipated, the ability to treat early stage lung cancer with SBRT/HIGRT is rapidly being incorporated into most Radiation Oncology facilities here in the United States.

SBRT/HIGRT in the setting of metastatic disease
SBRT/HIGRT is being used increasingly in the setting of metastatic disease. Initially, it was integrated as an ablative approach with a goal of tumor sterilization in oligometastatic disease with minimal morbidity and good long-term clinical outcome in these highly selected patients.(28-30) Subsequently, data have emerged that SBRT provides effective palliation in the metastatic setting for palliation of bone, lung, liver, and subcutaneous/nodal metastases with minimal morbidity.(28, 29, 31-33) As such, SBRT is being increasing utilized in the metastatic setting for palliative intent.

SBRT/HIGRT and Immune Activation
There is emerging evidence that hypofractionated radiotherapy is immunostimulatory. In a recently published study in *Blood*, Lee et al demonstrated that the therapeutic effect with ablative hypofractionated radiotherapy was dependent upon activation of CD8+ T- lymphocytes.(34) Additionally, there are pre-clinical data to suggest that CTLA-4 blockade along with ablative radiotherapy to an index lesion can prevent metastatic dissemination of disease.(35, 36) Finally, there are clinical data to suggest that SBRT/HIGRT provides greater local control and a reduction in regional and distant dissemination of disease when compared with surgery alone in NSCLC. One hypothesis to explain these surprising data is that the immunostimulatory effect of SBRT/HIGRT results in improved control of disease and prevention of spread.(37)

Radiation with PD-L1 blockade: We present a patient with widely metastatic Kras G12F mutant lung adenocarcinoma whose tumor had an increased expression of PD-L1. This patient had failed multiple therapies and had received radiation to a painful soft tissue metastasis. Four weeks after irradiation, she enrolled in a trial of MPDL3280A, and after receipt of 3 doses, demonstrated complete resolution of multiple metastases on (see Figure 2). This response has persisted at 1 year. Interestingly, following her 12th dose of MPDL3280A, the patient developed autoimmune manifestations of vitiligo as well as fasciitis (Fig. 3). This is consistent with a report by Deng et al in mouse models that administration of anti-PDL-1 antibody enhanced the effects of radiation through a cytotoxic T-cell dependent mechanism, providing rationale for combining radiation with immune modulators such as PDL-1 antibody therapy(4). A similar abscopal reaction with ipilimumab has been reported in patients as well (38). While the optimal radiation regimens for harnessing the proimmunogenic effects of radiation remain to be defined, pre-clinical data suggests that the ability of radiation to promote anti-tumor immunity may be dependent on the dose and fractionation employed. Animal models have also demonstrated a correlation between vitiligo and resistance to re-challenge with tumor after immune checkpoint blockade

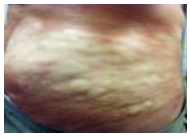


Fig 4: Hypo-pigmentation and fasciitis in previously radiated area following MPDL280.

Ablative radiotherapy doses has been shown to result in a greater degree of stromal/vascular damage and increased apoptosis of tumor cells (39), which may ultimately lead to an environment of enhanced antigen presentation. Animal models support this theory and suggest that a threshold likely exists in regard to the radiation fraction size necessary to induce an optimal immune response with ablative doses generating greater immunostimulatory effects as compared to conventional radiation doses (34), (40) .

Clinically, the ability to deliver ablative doses to tumors with acceptable toxicity has become possible over the past decade with technologic advancements in image guidance and radiation dose delivery. HIGRT/SBRT takes advantage of these advances in technology and allows for precise delivery of ablative doses with high rates of local control for both localized lung cancer as well as oligo-metastatic disease. In the setting of early-stage lung cancer, phase I/II trials demonstrate that the use of ablative radiation schema yields improved survival as compared to conventional fractionated treatment with local control rates comparable to surgical resection (41)

Radiation with nelfinavir in solid tumors:
Based upon our pre-clinical data, we performed a phase I/II trial of nelfinavir with concurrent chemoradiotherapy in patients with inoperable locally advanced NSCLC. The objective of the phase I trial was to determine dose-limiting toxicities (DLT) and the maximally tolerated dose of nelfinavir in combination with concurrent chemoradiotherapy (CT-RT) in locally advanced non-small cell lung cancer (NSCLC). We administered nelfinavir according to the following schema dose Level (DL) 1: 625mg PO BID, DL2:1250mg PO BID was given for 7 to 14 days prior to and concurrently with concurrent CT-RT to patients (pts) with biopsy confirmed IIIA or IIIB unresectable NSCLC. Five patients were treated at DL1; 7 patients were treated at DL2. Patients were treated with concurrent CT-RT to a dose of 66.6Gy. DLTs were defined as any treatment related Grade 4 hematologic toxicity requiring a break in therapy or non-hematologic Grade 3 or higher toxicity except esophagitis and pneumonitis. Sixteen patients were enrolled and 12 patients were treated with nelfinavir and concurrent chemoradiotherapy. No DLTs have been observed at either dose level. The maximum tolerated dose of

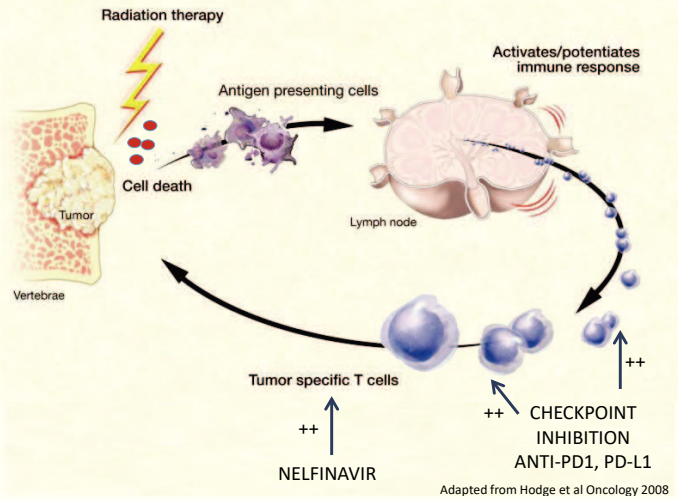


Figure 5 Mechanism of synergism between high-dose radiotherapy, nelfinavir, and checkpoint inhibition to achieve durable anti-tumor immune memory (Adapted from Hodge et al Oncology 2008)

Nelfinavir was therefore 1250 mg PO BID. Six patients experienced Grade 4 leukopenia. One patient experienced grade 4 thrombocytopenia. Median follow-up for all 12 patients was 31.6 months and for survivors is 23.5 months. Nine of the 12 patients had evaluable post-treatment PET/CT with metabolic response as follows: overall response: 9/9 (100%); complete response: 5/9 (56%); partial response 4/9 (44%). The median survival for all patients was 22.3 months. We concluded that nelfinavir administered with concurrent CT-RT is associated with acceptable toxicity in stage IIIA/IIIB NSCLC. The metabolic response and tumor response data suggest that nelfinavir has promising activity in this disease.(42) We have since proceeded with the phase II expansion with an additional 24 patients at the phase II dose of Nelfinavir and seen a promising median survival of 40 months which compares favorably with historical controls. Clinical trials are underway in a variety of tumor types with combination of radiation and nelfinavir. In general, these trials have all demonstrated promising clinical response rates without an increase in adverse events.(43-45) Furthermore, a clinical trial of nelfinavir in patients with metastatic treatment-refractory solid tumor demonstrated a promising response rate with 36% having stable disease for over six months, potentially suggestive of potential immune-augmentation by nelfinavir.(46)

1.4 Reference Therapy: PD-1 and PD-L1 Inhibitors

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [7; 8]. The structure of murine PD-1 has been resolved [9]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorization of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade [7; 10; 11; 12]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [13; 14]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells [15; 16]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [17]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [18; 19; 20; 13]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues [13]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL) [21]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

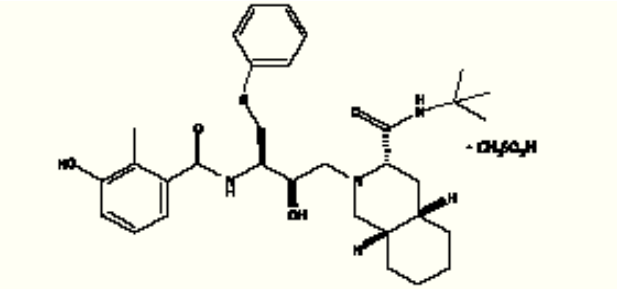
The primary objective of this clinical trial is to determine whether hypofractionated radiotherapy and PI-3*k* inhibition using Nelfinavir can improve upon standard-of-care immunotherapy by targeting the PD-1 receptor in melanoma, renal cell carcinoma, and non-small cell lung cancer. Since the time of this protocol's original development, the Food and Drug Administration has rapidly given approval to additional drugs that belong to the same class as nivolumab (PD1/PD-L1 inhibitors) with *some overlapping* clinical indications for melanoma, non-small cell lung cancer, and renal cell carcinoma. These drugs currently include pembrolizumab [49-52] for melanoma and NSCLC, atezolizumab [53-60] for NSCLC, with FDA-approval pending for durvalumab [61-64]. These drugs all have similar clinical efficacy

and toxicity profiles to nivolumab [65-69] in the setting of melanoma (response rates 30-40%) [50, 52], non-small cell lung cancer (response rates 30-40%) [49, 53], and renal cell carcinoma (~25%) [70]. These drugs have varying treatment schedules allowing the physician and patient greater flexibility to tailor the immunotherapy regimen to their needs and circumstances.

Currently, there is no direct comparison data to indicate the superiority of one PD1/PDL1 antibody over another. The available agents are considered equivalent in their applicable settings. There is no rigid order of prioritization. In the absence of other factors, our institution plans to select pembrolizumab as the first-choice agent, due to its preferred 3-week scheduling over the 2-week scheduling of nivolumab, for melanoma and NSCLC patients enrolled on this clinical trial. Pembrolizumab and nivolumab are both preferred over atezolizumab because they have been more extensively studied and characterized. Certain factors arise that may lead to the selection of nivolumab (for melanoma or NSCLC) or atezolizumab (NSCLC), including a patient's individual insurance approval as well as the continuation of previously-started PD1 therapy among patients in the PD1 refractory cohort. *See Section 3.1 Immunotherapy Reference Therapy*

Nelfinavir: There are ample data available regarding the use of NFV in humans [37]. The following is an excerpt of the key features.

VIRACEPT® (nelfinavir mesylate) is an inhibitor of the human immunodeficiency virus (HIV) protease. VIRACEPT Tablets are available for oral administration as a light blue, capsule-shaped tablet with a clear film coating in 250 mg strength (as NFV free base) and as a white oval tablet with a clear film coating in 625 mg strength (as NFV free base). Each tablet contains the following common inactive ingredients: calcium silicate, crospovidone, magnesium stearate, hypromellose, and triacetin. In addition, the 250 mg tablet contains FD&C blue #2 powder and the 625 mg tablet contains colloidal silicon dioxide. VIRACEPT Oral Powder is available for oral administration in 50 mg/g strength (as NFV free base) in bottles. The oral powder also contains the following inactive ingredients: microcrystalline cellulose, maltodextrin, dibasic potassium phosphate, crospovidone, hypromellose, aspartame, sucrose palmitate, and natural and artificial flavor. The chemical name for nelfinavir mesylate is [3*S*-[2(2*S**, 3*S**), 3a,4ab,8ab]]-*N*-(1,1-dimethylethyl)decahydro-2-[2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4- (phenylthio)butyl]-3-isoquinoline carboxamide mono-methane sulfonate (salt) and the molecular weight is 663.90 (567.79 as the free base). Nelfinavir mesylate has the following structural formula:



Nelfinavir mesylate is a white to off-white amorphous powder, slightly soluble in water at pH 4 and freely soluble in methanol, ethanol, 2-propanol and propylene glycol.

Pharmacokinetics

The pharmacokinetic properties of NFV were evaluated in healthy volunteers and HIV-infected patients; no substantial differences were observed between the two groups.

Absorption: Pharmacokinetic parameters of NFV (area under the plasma concentration-time curve during a 24-hour period at steady-state [AUC24], peak plasma concentrations [Cmax], morning and evening trough concentrations [Ctrough]) from a pharmacokinetic study in HIV-positive patients after multiple dosing with 1250 mg (five 250 mg tablets) twice daily (BID) for 28 days (10 patients) and 750 mg (three 250 mg tablets) three times daily (TID) for 28 days (11 patients) are summarized in Table 1.

Table 1
Summary of a Pharmacokinetic Study in HIV-positive Patients with Multiple Dosing of 1250 mg BID for 28 days and 750 mg TID for 28 days

Regimen	AUC_{24} mg•h/L	C_{max} mg/L	C_{trough} Morning mg/L	C_{trough} Afternoon or Evening mg/L
1250 mg BID	52.8 ± 15.7	4.0 ± 0.8	2.2 ± 1.3	0.7 ± 0.4
750 mg TID	43.6 ± 17.8	3.0 ± 1.6	1.4 ± 0.6	1.0 ± 0.5

data are mean ± SD

The difference between morning and afternoon or evening trough concentrations for the TID and BID regimens was also observed in healthy volunteers who were dosed at precisely 8- or 12-hour intervals. In healthy volunteers receiving a single 1250 mg dose, the 625 mg tablet was not bioequivalent to the 250 mg tablet formulation. Under fasted conditions (n=27), the AUC and Cmax were 34% and 24% higher, respectively, for the 625 mg tablets. In a relative bioavailability assessment under fed conditions (n=28), the AUC was 24% higher for the 625 mg tablet; the Cmax was comparable for both formulations. In healthy volunteers receiving a single 750 mg dose under fed conditions, NFV concentrations were similar following administration of the 250 mg tablet and oral powder.

Effect of Food on Oral Absorption: Food increases NFV exposure and decreases NFV pharmacokinetic variability relative to the fasted state. In one study, healthy volunteers received a single dose of 1250 mg of VIRACEPT 250 mg tablets (5 tablets) under fasted or fed conditions (three different meals). In a second study, healthy volunteers received single doses of 1250 mg VIRACEPT (5 x 250 mg tablets) under fasted or fed conditions (two different fat content meals). The results from the two studies are summarized in Table 2 and Table 3, respectively.

Table 2
Increase in AUC, C_{max} and T_{max} for Nelfinavir in Fed State Relative to Fasted State Following 1250 mg VIRACEPT (5 x 250 mg tablets)

Number of Kcal	% Fat	Number of subjects	AUC fold increase	C_{max} fold increase	Increase in T_{max} (hr)
125	20	n=21	2.2	2.0	1.00
500	20	n=22	3.1	2.3	2.00
1000	50	n=23	5.2	3.3	2.00

Table 3
Increase in Nelfinavir AUC, C_{max} and T_{max} in Fed Low Fat (20%) versus High Fat (50%) State Relative to Fasted State Following 1250 mg VIRACEPT (5 x 250 mg tablets)

Number of Kcal	% Fat	Number of subjects	AUC fold increase	C_{max} fold increase	Increase in T_{max} (hr)
500	20	n=22	3.1	2.5	1.8
500	50	n=22	5.1	3.8	2.1

NFV exposure can be increased by increasing the calorie or fat content in meals taken with VIRACEPT. A food effect study has not been conducted with the 625 mg tablet. However, based on a cross-study comparison (n=26 fed vs. n=26 fasted) following single dose administration of NFV 1250 mg, the magnitude of the food effect for the 625 mg NFV tablet appears comparable to that of the 250 mg tablets. VIRACEPT should be taken with a meal.

Distribution: The apparent volume of distribution following oral administration of NFV was 2-7 L/kg. NFV in serum is extensively protein-bound (>98%).

Metabolism: Unchanged NFV comprised 82-86% of the total plasma radioactivity after a single oral 750 mg dose of 14C-NFV. *In vitro*, multiple cytochrome P-450 enzymes including CYP3A and CYP2C19 are responsible for metabolism of NFV. One major and several minor oxidative metabolites were found in plasma. The major oxidative metabolite has *in vitro* antiviral activity comparable to the parent drug.

Elimination: The terminal half-life in plasma was typically 3.5 to 5 hours. The majority (87%) of an oral 750 mg dose containing 14C-NFV was recovered in the feces; fecal radioactivity consisted of numerous oxidative metabolites (78%) and unchanged NFV (22%). Only 1-2% of the dose was recovered in urine, of which unchanged NFV was the major component.

Special Populations

Hepatic Insufficiency: The multi-dose pharmacokinetics of NFV has not been studied in HIV-positive patients with hepatic insufficiency.

Renal Insufficiency: The pharmacokinetics of NFV has not been studied in patients with renal insufficiency; however, less than 2% of NFV is excreted in the urine, so the impact of renal impairment on NFV elimination should be minimal.

Gender and Race: No significant pharmacokinetic differences have been detected between males and females. Pharmacokinetic differences due to race have not been evaluated.

1.5 Dose Rationale

The delivery of the selected PD1 or PD-L1 immunotherapy will be carried out according to the FDA-approved dose and schedule.

Nelfinavir will be given at a dose of 1250 mg by mouth twice daily, with meals. This dosing is based upon the results of our phase I trial (47) and is the FDA-approved dose. All subjects will begin taking daily oral nelfinavir 7 to 14 days prior to the start of PD-1/PDL1 immune checkpoint inhibitor. Tthe 1-week range allows flexibility in scheduling the first dose of PD1/PDL1 immune checkpoint inhibitor. Nelfinavir will be given for a total of 12 weeks inclusive of the run-in period prior to immunotherapy. This corresponds to the duration of nelfinavir administration in the phase I trial. (47) Subjects will not receive any additional nelfinavir after 12 weeks, or 3 full bottles of Nelfinavir. The 7-14 days of nelfinavir prior to PD1/PDL1 immune checkpoint inhibitor is based on the known pharmacokinetics of the drug that has shown suppression of PI-3 kinase within three days after the administration of nelfinavir (see background information). Subjects will be asked to maintain a drug diary to assess compliance with administration of nelfinavir.

1.6 Other Agents

Radiation therapy is standard of care palliative treatment for patients with lung cancer, melanoma, or renal cell carcinoma presenting with focal symptomatic disease, oligometastatic disease, or progressive disease with impending functional consequences, such as airway or spinal canal encroachment.

Justification for combined modality approach

There is both pre-clinical and clinical rationale for exploiting the abscopal reaction in patients who have been treated with anti-PD-L1 therapy, nelfinavir, and radiation. Mounting data demonstrate that radiation can induce an effective immune response to tumors. The mechanisms of cooperation and improved disease control have not been clearly established but may include: (1) enhanced immunogenic antigen expression; (2) stimulation of cytokines release; and (3) increase in the permeability of the blood brain barrier for active agents to reach the metastatic lesions. Blockade of PD-1/PD-L1 by the FDA-approve PD1/PDL1 immune checkpoint inhibitor checkpoint inhibitor prior to radiotherapy may enhance the native lymphocyte response. The abscopal effect is a rare phenomenon of tumor regression at sites distant from an irradiated site. It has been observed in animal studies and humans following the administration of PDL1 antibodies. Nelfinavir-induced MDSC suppression in pre-clinical models was optimal when administered concurrently with PD-L1 blockade. The optimal schedule for combining these modalities together is unknown.

Justification for Hypofractionated Radiotherapy

While the optimal radiation regimens for harnessing the proimmunogenic effects of radiation remain to be defined, pre-clinical data suggests that the ability of radiation to promote anti-tumor immunity may be dependent on the dose and fractionation employed. Ablative radiotherapy doses have been shown to result in a greater degree of stromal/vascular damage and increased apoptosis of tumor cells (39), which may ultimately lead to an environment of enhanced antigen presentation. Animal models support this theory and suggest that a threshold likely exists in regard to the radiation fraction size necessary to induce an optimal immune response with ablative doses generating greater immunostimulatory effects as compared to conventional radiation doses (34) (40) . Clinically, the ability to deliver ablative doses to tumors with acceptable toxicity has become possible over the past decade with technologic advancements in image guidance and radiation dose delivery. Stereotactic body radiotherapy

(SBRT/HIGRT) takes advantage of these advances in technology and allows for precise delivery of ablative doses with high rates of local control for both localized lung cancer as well as oligometastatic disease. In the setting of early-stage lung cancer, phase I/II trials demonstrate that the use of ablative radiation schema yields improved survival as compared to conventional fractionated treatment with local control rates comparable to surgical resection.

1.7 Risks/Benefits

Please see informed consent form.

2.0 OVERVIEW OF CLINICAL TRIAL

2.1 Study Objectives

2.1.1 Primary Objective:

The primary objective of the study is to evaluate the response rate (complete (CR) or partial (PR) response, confirmed and unconfirmed) by irRECIST 1.1 in:

Patients with:

- Non-small cell lung cancer,
- Melanoma, or
- Renal cell carcinoma that is either naïve to or refractory to anti-PD-L1 or PD-L1 therapy

Who are treated with:

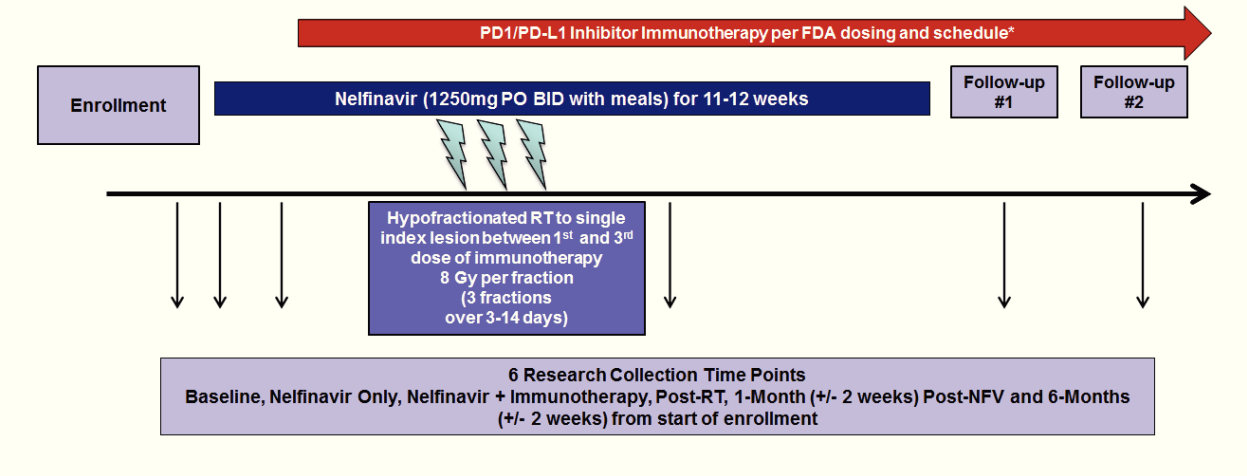
- Hypofractionated radiotherapy,
- Anti-PD-1/PD-L1 therapy and
- Nelfinavir

2.1.2 Secondary Objectives:

- To assess the safety and tolerability of the regimen as determined by the rate of grade 4 hepatoxicity
- To evaluate the frequency and severity of toxicities by CTCAE 5.0 attributed to treatment
- To evaluate progression-free survival within each disease and prior treatment cohort
- To evaluate overall survival within each disease and prior treatment cohort
- To evaluate the association between response and smoking status, underlying genetic mutations if known (e.g.: Kras, BRAF) circulating cfDNA, circulating tumor cells, PDL-1 expression in tumor and peripheral blood T cell receptor repertoire by sequencing within each disease and prior treatment cohort.

2.2 Study Design

This is a multi-cohort phase II trial of nelfinavir with hypofractionated radiotherapy and immunotherapy in advanced non-small cell lung carcinoma (NSCLC), melanoma, and renal carcinoma (RCC). The cohorts will be based upon histology (Melanoma vs NSCLC vs RCC) and prior immune checkpoint therapy (naive vs refractory).



**** See Selection Pathway Section 3.1***

2.3 Endpoints

2.3.1 **Primary Endpoint:** Best objective Response (complete or partial, confirmed or unconfirmed) by irRECIST 1.1. Patients not known to have a response will be coded as non-responders.

2.3.2 **Secondary Endpoint:**

- Overall survival (OS): OS is defined as the duration of start of study treatment to death due to any cause. OS for patients last known to be alive will be censored at the date of last contact.
- Progression-free survival (PFS): PFS is defined as the duration from start of treatment to progression by RECIST 1.1, symptomatic deterioration, or death due to any cause. PFS for patients last known to be alive and progression-free will be censored at the date of last contact.
- Adverse Events by CTCAE 5.0
- Immune correlative studies including changes in T-cell repertoire

3.0 STUDY AGENT INFORMATION

3.1 Immunotherapy Reference Therapy

PD1/PDL1 immune checkpoint inhibitor will delivered intravenously per standard of care.

Keytruda® (Pembrolizumab) FDA Approved Dosage and Administration

Metastatic Melanoma	200 mg intravenously over 30 minutes Every 3 weeks
Metastatic Non-Small Cell Lung Cancer	200 mg intravenously over 30 minutes Every 3 weeks

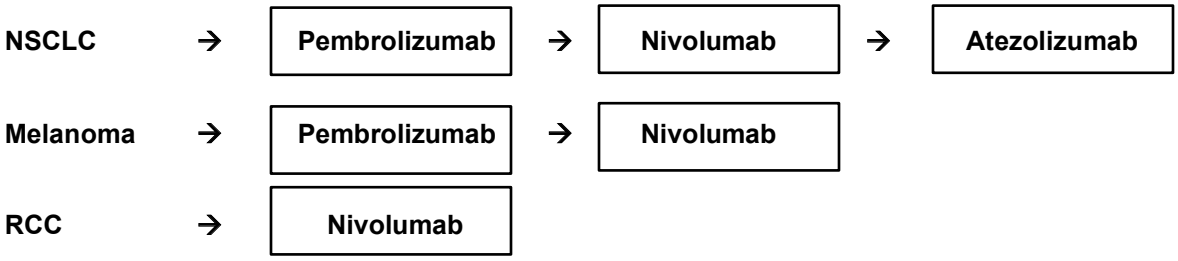
Opdivo® (Nivolumab) FDA Approved Dosage and Administration

Metastatic Melanoma	240 mg every 2 weeks or 480 mg every 4 weeks, intravenously over 30 minutes
Metastatic Non-Small Cell Lung Cancer	240 mg every 2 weeks or 480 mg every 4 weeks, intravenously over 30 minutes
Advanced Renal Cell Carcinoma	240 mg every 2 weeks or 480 mg every 4 weeks, intravenously over 30 minutes

Tecentriq® (Atezolizumab) FDA Approved Dosage and Administration

Metastatic Non-Small Cell Lung Cancer	1,200 mg intravenously over 60 minutes Every 3 weeks

Selection Pathway: In order of site preference



3.2 Receipt of Drug Supplies

Nelfinavir will be bought from commercial supply and stored as needed by Investigational Drug Services.

3.3 Dispensing of Study Drug

The drug will be dispensed by the UW and/or SCCA IDS.

3.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug dispensed, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

4.0 SUBJECT ELIGIBLITY

4.1 Inclusion Criteria

4.1.1 Disease eligibility and Stage

- Histologically confirmed diagnosis of melanoma, NSCLC, or renal carcinoma.
- Previously treated or previously untreated stage IV melanoma, stage IV or recurrent lung cancer, and metastatic renal cancer by AJCC staging criteria
- Presence of a lesion that is suitable for hypofractionated radiotherapy

4.1.2 Disease measurement specifications

4.1.3 **Subjects must have measurable disease by RECIST criteria independent of the lesion to be irradiated.** Prior checkpoint inhibitor immunotherapy or chemotherapy is allowed as long as the last dose was received >14 days prior to enrollment.

4.1.4 Age $\geq$ 18

4.1.5 ECOG 0-2 (see Appendix 14.2)

4.1.6 Acceptable marrow function and hematologic indices for PD1/PDL1 immune checkpoint inhibitor and nelfinavir as per standard of care.

4.1.7 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Subjects who have had immunotherapy, chemotherapy, or radiation therapy within 14 days (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.

4.2.2 Subjects may not be receiving other investigational agents.

4.2.3 Patients with untreated/active brain metastases as documented by CT or MRI within 2 months of study enrollment. By active brain metastases- we mean- actively symptomatic brain metastases requiring steroids.

4.2.4 Allergy or intolerance to nelfinavir or selected PD1/PDL1 immune checkpoint inhibitor.

4.2.5 Patients requiring steroids or other immunosuppressive therapy. Low-dose or topical steroids are allowable if being used as replacement therapy.

4.2.6 Patients receiving anti-retroviral therapy or other agents that are contra-indicated with nelfinavir due to drug-drug interactions.*

4.2.7 Pregnant or lactating patients.

4.2.8 Prior radiation that precludes delivery of hypofractionated radiotherapy.

* For a study regarding the safety and efficacy of high dose nelfinavir on patients with Kaposi's Sarcoma (KS), exclusion criteria included participants who were receiving any "strong inhibitors or inducers of cytochrome P450, family 3, subfamily A (CYP3A) or cytochrome P450, family 2, subfamily C, polypeptide 19 (2C19)"

Strong Inhibitors of CYP3A4:

- Antibiotics: clarithromycin, erythromycin, telithromycin, troleandomycin
- HIV: non-nucleoside reverse transcriptase inhibitors (delavirdine, nevirapine), protease inhibitors (ritonavir, indinavir, lopinavir/ritonavir, saquinavir), cobicistat-boosted antiretrovirals (e.g., elvitegravir); NOTE: Clinical trials have demonstrated that there are no clinically significant drug-drug interactions between nelfinavir and the following antiretrovirals:

efavirenz (strong CYP3A4 inhibitor), etravirine (strong CYP3A4 inhibitor); therefore, these antiretrovirals will not be excluded.

- Antifungals: itraconazole, ketoconazole, voriconazole, fluconazole, posaconazole
- Antidepressants: nefazodone
- Antidiuretic: conivaptan
- GI: cimetidine, aprepitant
- Hepatitis C: boceprevir, telaprevir
- Miscellaneous: seville oranges, grapefruit, or grapefruit juice and/or pomelos, star fruit, exotic citrus fruits, or grapefruit hybrids.

Strong Inducers of CYP3A4:

- Glucocorticoids: cortisone (> 50 mg), hydrocortisone (> 40 mg), prednisone (> 10 mg), methylprednisolone (> 8 mg), dexamethasone (> 1.5 mg)
- Anticonvulsants: phenytoin, carbamazepine, primidone, phenobarbital and other enzyme inducing anti-convulsant drugs (EIACD)
- Antibiotics: rifampin (rifampicin), rifabutin, rifapentine
- Miscellaneous: St. John's Wort, modafinil

Strong Inhibitors of CYP2C9:

- Antifungals: fluconazole; lists including medications and substances known or with the potential to interact with the CYP3A or 2C19

4.3 Criteria for Removal/Withdrawal from Treatment

4.3.1 Subjects may be removed from this study at any time at their discretion. Subjects may also be removed from this protocol if they develop any untoward side effects from the study medication. In addition there are stopping rules in place for lack of efficacy and excessive toxicity as detailed in the statistical section.

4.3.2 Disease progression will be clinically determined by the principal investigator. Patients who show disease progression will be taken off of the study.

4.3.3. Extraordinary Medical Circumstances. If at any time the constraints of this protocol are detrimental to the subject's health, the subject will be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.

5.0 SUBJECT REGISTRATION

Subjects will be registered by the FHCRC/UW Study Coordinator and entered into OnCore at seattlectms.org. A complete, signed, study consent and HIPAA consent and documentation of consent are required for registration.

6.0 TREATMENT PLAN

Treatment will be administered on an outpatient basis. All study treatment will be administered at the University of Washington Medical Center or Seattle Cancer Care Alliance. Immunotherapy may be continued at the subject's local institution once the active study period is over. The active study period is defined as the concurrent nelfinavir and radiation therapy.

6.1 Nelfinavir treatment dosage and administration

Nelfinavir will be available in Investigational Drug Services (IDS) at the University of Washington and Seattle Cancer Care Alliance. When a subject is enrolled, the drug will be obtained and dispensed from IDS. It is stored at room temperature. Nelfinavir will be self-administered at a dose of 1250 mg PO BID and the patient will administer at home. This dosing is based upon the results of our phase I trial. (47) All subjects will begin taking daily oral nelfinavir 7 to 14 days prior to the start of PD1/PDL1 immune checkpoint inhibitor. Nelfinavir will be continued for a total of 11-12 weeks, depending on when Nelfinavir was initiated and when the first dose of PD1/PDL1 immune checkpoint inhibitor is. This corresponds to the duration of nelfinavir administration in the phase I trial. (47) Subjects will not receive any additional nelfinavir after the 12th week of nelfinavir, or a total of 3 bottles. The 7-14 days of nelfinavir prior to PD1/PDL1 immune checkpoint inhibitor commencement are based on the known pharmacokinetics of the drug that has shown suppression of PI-3 kinase within three days after the administration of nelfinavir (see background information). Subjects will be asked to maintain a drug diary to assess compliance with administration of nelfinavir (appendix 14.3)

6.2 Hypofractionated Radiotherapy

Hypofractionated radiotherapy typically involves fewer fractions over fewer days but each fraction involves a higher dose, compared with conventional radiation techniques. The goal of HIGRT/SBRT in this setting is to deliver appropriate tumor directed palliation while minimizing exposure of surrounding normal tissues. The dose used to treat a given tumor will be based on the location of the lesion, as the organs at risk surrounding the lesion are likely to dictate the risk of normal tissue toxicity. The study will exclude irradiation of liver metastases as an added precaution.

Patients will undergo a 3 fraction HIGRT/SBRT regimen, treatment commencing between 1st and before 3rd cycle of PD1/PDL1 immune checkpoint inhibitor. Dose and fractionation will be determined per standard clinical practice and will be dependent on the location of the treated site and adjacent organs at risk.

Either 3D-conformal radiotherapy or intensity-modulated radiotherapy (including volumetric arc radiotherapy [VMAT]) is acceptable planning techniques. Planning techniques may differ for each lesion to be treated provided that tumor motion is properly accounted for with each technique when the target is near the thorax region (i.e. lung or liver). Daily image guidance is required for this study.

6.2.1 Dose and Fractionation

Patients will undergo a 3 fraction HIGRT/SBRT regimen over 3-14 days.

The 3 fraction regimen will employ a fraction size of 8 Gy per fraction, however a dose reduction to 6 Gy is allowed if 8 Gy is not achievable due to exceeding dose constraints

Dose rate: For the purpose of this study, dose rate utilized will be that which is commissioned by the manufacturer and the medical physics group for external beam radiotherapy delivery by the University of Washington, Department of Radiation Oncology. There will be no special dose rate modifications required for this study.

6.2.2 Localization, Simulation, and Immobilization Treatment Planning / Target Volumes

All patients will be immobilized in a custom designed device in the appropriate position to isolate the index lesion. All patients will then undergo CT-based treatment planning in the custom made immobilization device. The CT scan must capture the region of interest as well as surrounding organs at risk (OAR) with sufficient margin for treatment planning. The CT scan should be obtained with a uniform slice thickness of less than or equal to 3 mm throughout. The use of IV contrast is left to the discretion of the treating physician.

All lesions with potential for respiratory motion should be evaluated by appropriate means including 4D CT scan and/or implanted fiducial marker(s). Respiratory motion management including but not limited to active-breathing control, respiratory gating, and fiducial marker tracking, will be employed for qualifying patients per standard clinical practice.

Daily image guidance will be employed for target localization with volumetric imaging (cone- beam CT).

6.2.3 Target Volumes

The gross tumor volume (GTV) is defined as all known gross disease encompassing the selected index lesion as visualized the planning CT scan and aided by additional diagnostic imaging studies (PET/CT or MRI). The use of additional diagnostic imaging studies is dependent on the location of the index lesion and is left to the discretion of the treating physician. An internal gross tumor volume (IGTV) is defined for mobile index lesions at the discretion of the treating physician. A 4-D CT scan will be acquired in order to account for the motion of the lesion during. The IGTV will be defined as the union of the visualized index lesion on all gated CT data sets.
The clinical target volume (CTV) will equal the GTV/IGTV; there will be no margin added for microscopic extension.
The planning target volume (PTV) will be defined as per the convention for photon beam radiotherapy. A 3-dimensional margin will be created on the GTV or IGTV (if available) to allow for daily set-up variance.

6.2.4 Normal Structures

Organ at risk volume (OAR) is contoured as visualized on the planning CT or MR scan. Planning PAR is the OAR expanded for setup uncertainty or organ motion. The physician will contour the OAR. The dosimetrist will create the PAR by expanding the OAR by 2-3 mm, depending on the situation.

6.2.5 Treatment Planning

Multiple planning techniques may be employed to deliver HIGRT/SBRT to the index lesion, including static 3D coplanar and/or non-coplanar beam arrangements as well as dynamic conformal arcs or IMRT.

Three-dimensional coplanar or non-coplanar beam arrangements will be custom designed for each case to deliver highly conformal prescription dose distributions. Non-opposing, noncoplanar beams are preferable. Typically, ≥ 10 beams of radiation will be used with roughly equal weighting. Generally, more beams are used for larger lesion sizes.

For arc rotation techniques, a minimum of 340 degrees (cumulative for all beams) should be utilized. For arc rotation techniques, a minimum of 340 degrees (cumulative for all beams) should be utilized.

Critical Organ Doses: All critical organ dose-volume limits will be respected.
Planning Priorities: Every attempt will be made to successfully satisfy all of the planning goals and OAR criteria without deviation. In some circumstances, it may not be possible to meet all the ideal criteria. In these cases, spinal cord, cauda equine, sacral plexus, and brachial plexus dose constraints must be respected over PTV coverage. In the case of other OAR constraints, which are not well validated, PTV coverage and OAR constraints must be balanced per clinical practice at the discretion of the treating physician.

6.3 Toxicities and Dosing Delays

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed on an ongoing basis for the development of toxicity according to the study calendar (See Appendix 14.4). Toxicity will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 5.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

6.3.1 Stopping rules for the study related to Grade 5 adverse events, or suspected hepatoxicity are detailed in section 11.0 Statistical Considerations Criteria for Nelfinavir Dosing

Nelfinavir is known as a well-tolerated drug. In our phase I clinical trials [47], there were no dose-limiting or unacceptable toxicities reported. If a patient is unable to tolerate the daily dose, he/she will indicate on his/her pill diary (see Appendix14.3) how many pills, if any, were taken that day. Efforts will be made by the study team to achieve 100% compliance by engaging in regular communication with the patient. Patients are instructed to record any symptoms they are feeling so that they can discuss it with their provider. All adverse events that are attributed as **certainly, probably, or possibly** related to nelfinavir will be recorded.

Patients will delay or discontinue treatment with Nelfinavir if they experience at least one adverse event, specified below, considered by the treating investigator to be **certainly, probably, or possibly** related to Nelfinavir treatment. Patients may continue PD1/PDL1 inhibitor Immunotherapy treatment if the criteria in 6.3.8 is not met.

The following criteria will be used to determine dosing delay, restarting doses, or discontinuing Nelfinavir treatment.

6.3.2 Criteria to delay Nelfinavir Dosing

Treatment-related Event	Action
Any ≥ Grade 3 Adverse Event related to NFV	Delay Nelfinavir dosing

6.3.3 Criteria to resume Nelfinavir dosing

- Restart Nelfinavir dosing if/when the adverse event(s) resolve(s) to < Grade 2 severity or returns to baseline within 1 month.
    - If the adverse event has not resolved within 1 month, permanently discontinue Nelfinavir dosing
    - Restart Nelfinavir at 625mg PO BID for 1 week.
    - Monitor if adverse event re-emerges.
    - Return to 1250mgPO BID after 1 week.

6.3.4 Criteria for permanent discontinuation of Nelfinavir

Treatment-related Event	Action
Any ≥ Grade 3 adverse event that does not improve to ≤ Grade 2 severity or return to baseline within 1 month.	Permanently discontinue Nelfinavir dosing
Any recurrence of ≥ Grade 3 adverse event that required previous Nelfinavir dosing delay	

6.3.5 Criteria for PD1/PDL1 inhibitor immunotherapy treatment

Patients may develop PD1/PDL1 immune checkpoint inhibitor-related toxicities that may require skipping doses or dose discontinuation. Some of these adverse events may be consistent with potentially drug-related immune-mediated phenomena; termed IRAEs.

Patients will delay or discontinue treatment with their selected PD1/PDL1 immune checkpoint inhibitor if they experience at least one adverse event, specified below, considered by the treating investigator to be **certainly, probably, or possibly** related to PD1/PDL1 immune checkpoint inhibitor treatment.

The following criteria will be used to determine dosing delay, restarting doses, or discontinuing PD1/PDL1 inhibitor immunotherapy.

6.3.6 Criteria to delay one dose of PD1/PDL1 inhibitor immunotherapy treatment

Treatment-related Event	Action
Any ≥ Grade 2 non-skin related adverse event (including IRAEs) except for laboratory abnormalities	Delay PD1/PDL1 inhibitor immunotherapy dosing
Any ≥ Grade 3 laboratory abnormality	
Any ≥ Grade 3 skin-related adverse event (including IRAEs) regardless of causality.	

6.3.7 Criteria to resume PD1/PDL1 inhibitor immunotherapy treatment

- Restart PD1/PDL1 inhibitor immunotherapy dosing if/when the adverse event(s) resolve(s) to ≤ Grade 1 severity or returns to baseline within 3 weeks of initial dose administration
- If the adverse event has resolved (to ≤ Grade 1 severity or returns to baseline), restart PD1/PDL1 inhibitor immunotherapy dosing at the next scheduled dosing time point per protocol.
- If the adverse event has not resolved in the protocol-specified dosing window (2 weeks [+/- 3 days], the next scheduled dose will be omitted.
- Patients with IRAEs who require steroid therapy with resolution to ≤ Grade 1 severity must be taking no more than 7.5mg of prednisone (or the equivalent) before resuming PD1/PDL1 inhibitor immunotherapy.

6.3.8 Criteria for permanent discontinuation of PD1/PDL1 inhibitor immunotherapy for Related Adverse Events

Event	Action
Any ≥ Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to ≤ Grade 1 severity within 2 weeks of starting therapy, OR, requires systemic treatment.	Permanently discontinue PD1/PDL1 inhibitor immunotherapy
Any ≥ Grade 3 bronchospasm or other hypersensitivity reaction.	

Any other ≥ Grade 3 non-skin related adverse event with the exception of events listed under “Exceptions to Permanent Discontinuation”	
Any ≥ Grade 4 laboratory abnormalities, except AST, ALT, or Total Bilirubin.[1] • AST or ALT>8xULN. • Total Bilirubin > 5 x ULN.	
Any other ≥ Grade 4 adverse event.	
Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the patient with continued dosing.	
Any motor neurologic toxicity ≥ Grade 3 regardless of causality.	
Any ≥ Grade 3 treatment related sensory neurologic toxicity.	
Patients who require high dose steroids, other immune suppressants or anti-TNF drug therapy for the management of immune related adverse events should have the PD1/PDL1 inhibitor immunotherapy permanently discontinued*.	

[1]Exception to permanent discontinuation of selected PD1/PDL1 inhibitor immunotherapy is made for laboratory abnormalities that are rapidly reversible, not life threatening, do not reflect underlying organ system dysfunction, and are not related to the study treatment, such as transient elevations of uric acid, hypocalcaemia, hypophosphatemia.

PD1/PDL1 inhibitor immunotherapy administration may be resumed in the following cases:

- Potentially reversible inflammation (< Grade 4), attributable to a local anti-tumor reaction and a potential therapeutic response. This includes inflammatory reactions at thoracotomy sites or at sites suspicious for, but not diagnostic of metastasis.
- Hospitalization for ≤ Grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.
- Patients with the following conditions where in the investigator’s opinion continuing study drug administration is justified:
    - Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy (e.g. hypothyroidism).
    - Ocular toxicity that has responded to topical therapy.

6.4 Immune-Related Adverse Events (irAEs): Definition, Monitoring, and Treatment

Blocking PD-L1 function may permit the emergence of auto-reactive T cells and resultant clinical autoimmunity. Rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis, and hypopituitarism are drug-related, presumptive autoimmune events, termed irAEs, noted in previous studies.

For the purposes of this study, an irAE is defined as an AE of unknown etiology associated with drug exposure and consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an AE an irAE.

Patients should be informed of and carefully monitored for evidence of clinically significant systemic irAE (e.g., systemic lupus erythematosus-like diseases) or organ-specific irAE (e.g., rash, colitis, uveitis, hepatitis or thyroid disease). If an irAE is noted, appropriate work-up should be performed, and steroid therapy may be considered if clinically necessary (see below).

Toxicities associated or possibly associated with PD1/PDL1 immune checkpoint inhibitor treatment should be managed according to standard medical practice. Additional tests, such as autoimmune serology or biopsies, may be used to determine a possible immunogenic etiology.

Although most irAEs observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications. Discontinuation of selected PD1/PDL1 immune checkpoint inhibitor may not have an immediate therapeutic effect and, in severe cases, immune related toxicities may require acute management with topical corticosteroids, systemic corticosteroids, mycophenolate, or tumor necrosis factor alpha (TNFα) inhibitors.

The primary approach to Grade 1 to 2 irAEs is supportive and symptomatic care with continued treatment with PD1/PDL1 immune checkpoint inhibitor; for higher-grade irAEs, PD1/PDL1 immune checkpoint inhibitor should be withheld and oral and/or parenteral steroids administered. Recurrent Grade 2 irAEs may also mandate withholding PD1/PDL1 immune checkpoint inhibitor or the use of steroids.

Assessment of the benefit risk balance should be made by the investigator, with consideration of the totality of information as it pertains to the nature of the toxicity and the degree of clinical benefit a given patient may be experiencing prior to further administration of PD1/PDL1 immune checkpoint inhibitor. The PD1/PDL1 immune checkpoint inhibitor should be permanently discontinued in patients with life threatening irAEs.

6.5 Concomitant Medications/Treatments

- Examples of supportive medications include acetaminophen, NSAIDs, antihistamines, and anti-diarrheals.
- Bisphosphonate use for bone metastasis is allowed.
- Herbal supplements must be approved by investigator.
- Concomitant antineoplastic therapy is prohibited.
- All medications need careful review for drug-drug interactions with Nelfinavir. Additional safety monitoring may be needed.

  Per the VIRACEPT (Nelfinavir Mesylate) package insert, Nelfinavir is contraindicated with drugs that are highly dependent on CYP3A for clearance and for which elevated plasma concentrations are associated with serious and/or life threatening events. Below is a table of drugs that are contraindicated with long-term VIRACEPT therapy. Drug-drug interactions will be evaluated in the context of the short-term (12 week) duration of administration of Viracept in this trial and drug regimens will be modified accordingly:

Drug Class	**Drugs Within Class That Are Contraindicated with VIRACEPT**	**Clinical Comment**
Alpha 1-adrenoreceptor antagonist	Alfuzosin	Potentially increased alfuzosin concentrations can result in hypotension.
Antiarrhythmics	Amiodarone, quinidine	Potential for serious and/or life-threatening cardiac arrhythmia
Antimycobacterial Agents	Rifampin	Plasma concentrations of nelfinavir can be reduced by concomitant use of rifampin. This may lead to loss of therapeutic effect and possible development of resistance to VIRACEPT or other coadministered antiretroviral agents.

Antipsychotics	Lurasidone Pimozide	Potential for serious and/or life-threatening reactions. Potential for serious and/or life threatening reactions such as cardiac arrhythmias.
Ergot Derivatives	Dihydroergotamine, ergotamine, methylergonovine	Potential for serious and/or life threatening reactions such as ergot toxicity characterized by peripheral vasospasm and ischemia of the extremities and other tissues.
GI Motility Agent	Cisapride	Potential for serious and/or life threatening reactions such as cardiac arrhythmias.
Herbal products	St. John's wort (Hypericum perforatum)	Plasma concentrations of nelfinavir can be reduced by concomitant use of the herbal preparation St. John's wort. This may lead to loss of therapeutic effect and possible development of resistance to VIRACEPT or other coadministered antiretroviral agents
HMG-CoA Reductase Inhibitors	Lovastatin, Simvastatin	Potential for serious reactions such as myopathy including rhabdomyolysis.
PDE5 Inhibitors	Sildenafil (Revatio®) [for treatment of pulmonary arterial hypertension]a	A safe and effective dose has not been established when used with nelfinavir. There is increased potential for sildenafilassociated adverse events (which include visual disturbances,
Sedative/Hypnotics	Triazolam, oral midazolam	Potential for serious and/or life threatening reactions such as prolonged or increased sedation or respiratory depression.

6.6 Duration of Therapy

Reference PD1/PDL1 inhibitor therapy ends at the time of progression (as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (irRECIST v1.1) by physical examination and radiographic assessment, primarily CT scan, immune response criteria and/or loss of clinical benefit as assessed by the investigator). The study period is defined from the time of consent through the follow-up period or end of service. The last follow-up appointment occurs 6-months after the last dose of nelfinavir. Patients will be seen prior to end of service as per standard of care. Additional endpoints will include overall survival and safety.

6.7 Rationale for Allowing Patients to Continue PD1/PDL1 immune checkpoint inhibitor Treatment until Loss of Clinical Benefit

Conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. Because of the potential for pseudo progression/tumor immune infiltration, this study will allow patients to remain on PD1/PDL1 immune checkpoint inhibitor after apparent radiographic progression, provided the benefit-risk ratio is judged to be favorable. Patients should be discontinued for unacceptable

toxicity or symptomatic deterioration attributed to disease progression as determined by the investigator after an integrated assessment of radiographic data, biopsy results (if available), and clinical status. Patients who show evidence of clinical benefit will be permitted to continue PD1/PDL1 immune checkpoint inhibitor after RECIST v1.1 criteria for progressive disease are met if they meet all of the following criteria:

- Evidence of clinical benefit as assessed by the investigator
- Absence of symptoms and signs (including worsening of laboratory values, e.g., new or worsening hypercalcemia) indicating unequivocal progression of disease
- No decline in ECOG performance status that can be attributed to disease progression
- Absence of tumor progression at critical anatomical sites (e.g., leptomeningeal disease) that cannot be managed by protocol-allowed medical interventions
- Patients for whom approved therapies exist must provide written consent to acknowledge deferring these treatment options in favor of continuing study treatment at the time of initial progression

The end of participation in the study may also be reached sooner if one of the following criteria applies:

- Inter-current illness that prevents further administration of treatment or follow-up
- Unacceptable adverse event(s)
- Patient voluntarily withdraws from treatment OR
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

6.8 Off Treatment Criteria

Patients will be removed from protocol therapy when any of the criteria listed in Section 6.3.8 applies. The source document will give the reason for ending protocol therapy and the date the patient was removed from treatment. All patients who discontinue treatment should comply with protocol specific follow-up procedures as outlined in Appendix 14.4. There are two exceptions to this requirement:

- Patient starts a new anti-cancer therapy and/or receives additional radiation treatment prior to follow-up procedures
- Patient withdraws consent for all study procedures or loses the ability to consent freely.

6.9 Duration of Follow-Up

Patients will be followed after completion or removal from protocol treatment at two protocol-mandated time points. The first follow-up will occur 1 month after last dose of Nelfinavir (+/- 2 weeks), while the second follow-up will occur 6 months from date of enrollment (+/- 2 weeks). All patients who discontinue treatment should comply with the procedures below unless they meet the exceptions in Section 6.8:

- Physical Exam; ECOG
- Adverse Events Monitoring
- Concomitant Medication Monitoring
- Response Assessment by irRECIST 1.1
- Research Blood Collection
- Optional tumor biopsy

Survival follow-up will continue for 2 years from enrollment through office visits (scheduled at clinically indicated intervals, will vary by patient) and by review of medical record. Subjects may be contacted by phone call every 6 months to determine vital status.

6.10 Off Study Criteria

Patients can be taken off study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation from study will be documented and may include:

- Patient withdraws consent (termination of treatment and follow-up)
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment;
- Patient is unable to comply with protocol requirements.
- Treating physician judges' continuation on the study would not be in the patient's best interest.
- Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event.
- Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study
- Lost to Follow-up. If a research subject cannot be located to document survival after a period of 2 years, the subject may be considered "lost to follow-up." All attempts to contact the subject during the two years must be documented.
- Termination of the study by the University of Washington.

6.11 Patient Replacement

A patient who is enrolled but did not receive any protocol therapy (one dose of PD1/PDL1 immune checkpoint inhibitor) is considered non-evaluable, and will be replaced.

6.12 Immunological Studies

Blood samples for immunologic correlated research will be collected at study intervals: baseline, nelfinavir only (i.e. nelfinavir run-in period), nelfinavir and first dose PD1/PDL1 immune checkpoint inhibitor (i.e. prior to second dose of PD1/PDL1 immune checkpoint inhibitor), post-radiation treatment, 1 month post-Nelfinavir, and 6 months from date of enrollment (see 14.4 Treatment Calendar). Samples will be labeled with the subject's de-identified study number and collection date, processed, frozen and stored. The blood will be kept within the Cancer Consortium.

7.0 ADVERSE EVENTS

7.1 Adverse Event Reporting

In accordance with institutional policy, all adverse events which in the opinion of the principal investigator are unexpected and related or possibly related to the research and serious or suggest that the research places research participants or others at greater risk of physical or psychological harm than was previously known or recognized be reported to the IRB within 10 calendar days of learning of the problem.

Definitions:

Adverse Event - Any harm or untoward medical occurrence in a research participant administered a medical product, medical treatment or procedure even if it does not necessarily have a causal relationship with the product, treatment, or procedure. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related.

Unexpected Adverse Event – An adverse event is "unexpected" when its nature (specificity), severity, or frequency are not consistent with (a) the known or foreseeable risk of adverse events associated with the research procedures described in the protocol-related documents, such as the IRB-approved research

protocol, informed consent document and other relevant sources of information such as product labeling and package inserts; and are also not consistent with (b) the characteristics of the subject population being studied including the expected natural progression of any underlying disease, disorder or condition any predisposing risk factor profile for the adverse event.

Serious Adverse Event (SAE) – Any adverse event occurring that results in any of the following outcomes:

- death
- a life-threatening adverse event (real risk of dying)
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity/or change in psychosocial status
- a congenital anomaly
- requires intervention to prevent permanent impairment of damage

In some instances there may be exceptions to hospitalization as an SAE. Hospital admissions for co-morbid conditions, tumor-related diagnostic procedures, or conditions unrelated to the study treatment are examples of what may be exceptions.

Attribution - The following are definitions for determining whether an adverse event is related to a medical product, treatment or procedure:

- An adverse event is "**related or possibly related to the research procedures** "if in the opinion of the principal investigator, it was more likely than not caused by the research procedures.

- Adverse events that are solely caused by an underlying disease, disorder or condition of the subject or by other circumstances unrelated to the research or any underlying disease, disorder or condition of the subject are not "related or possibly related."

- If there is any question whether or not an adverse event is related or possibly related, the adverse event should be reported.

The synergistic effects of PD1/PDL1 immune checkpoint inhibitor and nelfinavir are not fully studied; therefore all AEs will be recorded if provider is unable to rule out the adverse event as unrelated to both nelfinavir and PD1/PDL1 immune checkpoint inhibitor. Both the Cancer Consortium IRO Expedited Reporting Form for Unanticipated Problems or Noncompliance and the Adverse Event Reporting Form should be completed for all adverse events that meet the expedited reporting requirements. The forms should be mailed directly to the IRO (J2-100) no later than 10 calendar days after the Principal Investigator first becomes aware of the event. Submit only the original documents. All available information should be submitted.

7.2 Duration and Grade of Adverse Event Capture

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring after nelfinavir starts will be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.3 Adverse Event Grading

Toxicities will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Citing the website under this section in the protocol rather than printing the entire document is sufficient unless the study is modifying the criteria.

All CTC grades will be followed and reported unless noted in the protocol. For IND studies, FDA review of exceptions to AE reporting is recommended: note the duration of the AE reporting period and grades of AEs to be captured.

7.4 Investigator Reporting: Notifying the FHCRC Institutional Review Office

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the PI by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the CCIRB within 24 hours. The investigator will keep a copy of this SAE form on file.

Ramesh Rengan MD PhD Phone: 206-598-4110; Mobile: 206-890-7195; or via the UW Page operator

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the CCIRB

8.0 DATA AND SAFETY MONITORING PLAN

Protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCRC Scientific Review Committee (SRC) and the FHCRC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

In addition, protocol will be reviewed approximately every 6 months while the study is enrolling and as needed by an independent Data and Safety Monitoring Board (DSMB). DSMB responsibilities are outlined in the DSMB Charter. All DSMB members are completely independent of the trial and are in compliance with institutional policy regarding conflict of interest. The PI will be responsible for promptly reviewing the DSMB recommendations to decide whether to continue or terminate the trial, and to determine whether amendments to the protocol or changes in study conduct are required. As recommended by the DSMC in July 2019, the DSMB will convene after the re-opening of the trial to enrollment in 2019 or 2020 to review the 1st 2 or 3 patients enrolled (or within a pre-specified time frame- e.g. 3 months) on the protocol and will file a report for the DSMC to review. The DSMB will meet quarterly thereafter.

Institutional support of trial monitoring will be in accordance with the FHCRC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, FHCRC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCRC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

9.0 ASSESSMENT OF EFFICACY

9.1 Efficacy Parameters

This protocol will assess response according to irRECIST v1.1 and the immune-related response criteria as outlined by Nishino et al as outlined in section 4.2.(48)

9.2 Method and Timing

Patients will undergo their initial response assessment by CT scans between weeks 12-14 after initiation of -PD1/PDL1 immune checkpoint inhibitor, every 12-14 weeks thereafter until progression, or as clinically indicated per institutional standards. The response assessments will not be read in real time. The formal reads of the response assessments will be completed once there is a statistically valid endpoints assessment at the end of trial accrual.

9.3 Other Response Parameters

Immune correlative studies will be performed on bio-specimens collected and is outlined in the study schema and calendar. The time-points of blood collection will be at baseline, while on nelfinavir only, after the first dose of PD1/PDL1 immune checkpoint inhibitor, post-radiation treatment, 1 month (+/- 2 weeks) after taking Nelfinavir, and 6 months (+/- 2 weeks) from enrollment (see Appendix 14.4 Treatment Calendar).

10.0 DATA MANAGEMENT/CONFIDENTIALITY

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique patient number to assure subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents.

11.0 STATISTICAL CONSIDERATIONS

11.1 Sample Size with Power Justification

The primary objective of this study is to evaluate the response rate in patients with non-small cell lung cancer (NSCLC), melanoma, or renal cell carcinoma (RCC) treated with nelfinavir, radiation therapy and anti-PD-1/PD-L1 therapy. Evaluation of this investigational therapy will be done in disease-specific cohorts separately evaluated by patients naïve to anti-PD-L1/PD-L1 therapy and those with disease refractory to anti-PD-1/PD-L1 therapy.

The total sample size for this study is 20 patients in each of the 6 cohorts (3 disease type by 2 anti-PD-1/PD-L1 exposure groups) for a total of 120 patients evaluable for response. The expected accrual rate is 30 patients per year. The design within each cohort is a single arm, single stage design. The sample size justification for each of the cohorts is follows. Sample size calculations were done using this calculator: https://stattools.crab.org/Calculators/oneArmBinomial.html.

Non-Small Cell Lung Cancer (NSCLC):

1. Checkpoint Naïve: In this cohort a true response rate of 50% or greater would be considered evidence of activity whereas a response rate of 25% or less (based on historical data) would not be considered of interest. With 20 patients, this study has 86% power to rule out a response rate of 25%, at the 1-sided 0.10 level if the true response rate were 50%. The observation of at least 8 responses (8/20; 40% ORR) would be

considered evidence to rule out a response rate of 25%. The exact power and type I error for this design are 87% and 10%, respectively.

2. Checkpoint refractory: In this cohort a true response rate of 25% or greater would be considered evidence of activity whereas a response rate of 5% or less (based on historical data) would not be considered of interest. With 20 patients, this study has 91% power to rule out a response rate of 5%, at the 1-sided 0.10 level if the true response rate were 25%. The observation of at least 3 responses (3/20; 15% ORR) would be considered evidence to rule out a response rate of 5%. The exact power and type I error for this design are 91% and 7.6%, respectively.

Melanoma:

3. Checkpoint Naïve: In this cohort a true response rate of 68% or greater would be considered evidence of activity whereas a response rate of 43% or less (based on historical data) would not be considered of interest. With 20 patients, this study has 84% power to rule out a response rate of 43%, at the 1-sided 0.10 level if the true response rate were 68%. The observation of at least 11 responses (11/20; 55% ORR) would be considered evidence to rule out an rate of 43%. The exact power and type I error for this design are 93% and 19.5%, respectively.

4. Checkpoint refractory: In this cohort a true response rate of 40% or greater would be considered evidence of activity whereas a response rate of 15% or less (based on historical data) would not be considered of interest. With 20 patients, this study has 90% power to rule out a response rate of 15%, at the 1-sided 0.10 level if the true response rate were 40%. The observation of at least 5 responses (5/20; 25% ORR) would be considered evidence to rule out an response rate of 15%. The exact power and type I error for this design are 95% and 17%, respectively.

Renal Cell Carcinoma:

5. Checkpoint Naïve: In this cohort a true response rate of 50% or greater would be considered evidence of activity whereas a response rate of 25% or less (based on historical data) would not be considered of interest. With 20 patients, this study has 86% power to rule out a response rate of 25%, at the 1-sided 0.10 level if the true response rate were 50%. The observation of at least 8 responses (8/20; 40% ORR) would be considered evidence to rule out a response rate of 25%. The exact power and type I error for this design are 87% and 10%, respectively.

6. Checkpoint refractory: In this cohort a true response rate of 25% or greater would be considered evidence of activity whereas a response rate of 5% or less (based on historical data) would not be considered of interest. With 20 patients, this study has 91% power to rule out a response rate of 5%, at the 1-sided 0.10 level if the true response rate were 25%. The observation of at least 3 responses (3/20; 15% ORR) would be considered evidence to rule out a response rate of 5%. The exact power and type I error for this design are 91% and 7.6%, respectively.

11.2 Analysis Plan

The primary analysis within each cohort will done on patients who receive at least one dose of nelfinavir, and anti-PD-1/PD-L1 therapy and at least one fraction of radiation. The safety analysis population will include all patients who receive at least one dose of any of the study drugs.

Binary endpoints (response, toxicity) will be summarized as proportions with associated 90% Clopper-Pearson confidence intervals. With 20 patients per cohort, binary proportions can be estimated to within 18% with 90% confidence. Any toxicity with true prevalence of at least 10% is likely to be observed (with 88% probability).

The distribution of time-to-event endpoints will be estimated using the method of Kaplan-Meier to derive estimates for median times and percentages at landmark times (e.g. 6 months). Confidence intervals for median times will be estimated using the Brookmeyer-Crowley method and for landmark times will be calculated using Greenwood's formula and based on a log-log transformation applied on the survival function.

A logistic regression model will be used to assess the association between response and factors such as smoking within each cohort. These associations will be summarized by odds ratios and associated confidence intervals and p-values. A Cox regression model will be used to assess the association between time-to-event outcomes (PFS,OS) and factors within each cohort. Similarly, these associations will be summarized by hazard ratios and associated confidence intervals and p-values.

11.3 Safety Monitoring

Toxicities will be monitoring on an on-going basis. If a Grade 5 adverse event with attribution as possibly, probably, or likely related to treatment is observed, then accrual to the study will be placed on hold with review by the DSMB needed. Consideration for the event will include if the patient was naïve to anti-PD-1/PD-L1 therapy or previously-exposed to anti-PD-1/PD-1 therapy.

A key toxicity of concern is hepatoxicity, with grade 4 interpreted as a clinically significant event. Historical rates of Grade 3-4 hepatoxicity with PD-1/PD-L1 therapy are 5-30% and 3% with nelfinavir. For monitoring of this event, evidence suggesting that the observed rate of Grade 4 hepatoxicity exceed a true rate of 15% would result in temporary closure of the study with expedited DMSC review. Evidence to suggest this will be based on the lower bound of an 80% confidence interval excluding 15%, pooling all patients on trial (regardless of disease type and prior exposure to anti-PD-1/PD-L1) and assessed within enrollment cohorts. As such, accrual to the study will be placed on hold if 2/2-4, 3/5-8, 4/9-12, 5/13-17, 6/18-21, 7/22-26, 8/27-31, 9/32-36, 10/37-41, 11/42-46,12/47-51, 13/52-57, 14/58-62, 15/63-67, 16/68-73, 17/74-78, 18/79-84, 19/85-89, 20/90-95, 21/96-100, 22/101-106, 23/107-111, 24/112-117, or 25/118-120 patients are observed to have a Grade 4 hepatoxicity event.

11.4 Accrual

Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	8	10	18
Not Hispanic or Latino	42	60	102
Ethnic Category Total of All Subjects*	50	70	120
Racial Categories			
American Indian / Alaska Native	4	4	8

Asian	4	4	8
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	4	4	8
White	38	58	96
More Than One Race	0	0	0
Racial Categories: Total of All Subjects*	50	70	120

12.0 INVESTIGATOR OBLIGATIONS

The PI is responsible for the conduct of the clinical trial at the site and is responsible for personally overseeing the treatment of all study subjects. The PI must assure that all study site personnel, including sub-Investigators and other study staff members, adhere to the study protocol and to all applicable regulations and guidelines regarding clinical trials both during and after study completion.

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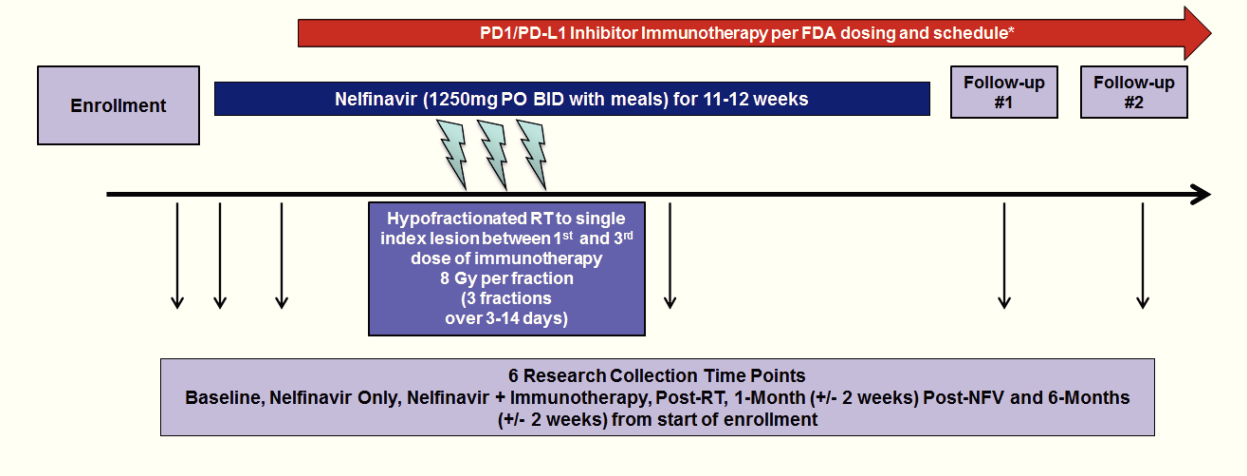
14.0 APPENDICES

14.1 Study Schema



14.2 ECOG Performance Status Scale

ECOG Performance Scale

GRADE	SCALE
0	Fully active, able to carry out all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

14.3 Patient Pill Calendar

Patient Pill Calendar

Patient ID #_____Patient Initials:_____Bottle #:_____

This is a calendar on which you are to record the time and number of tablets you take each day. You should take your scheduled dose of each pill. **Note the times and the number of tablets that you take each day.** If you develop any side effects, please record them and anything you would like to tell the doctor in the space provided. Bring any unused tablets and your completed pill calendar to your doctor's visits.

DAY	Date Month Day Year			Time pills taken AM	 PM	Number of pills taken AM	 PM	Use the space below to make notes about things you would like to tell the doctor (including unusual symptoms you experience, etc.)
1								
2								
3								
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14.4 Treatment Calendar

	Baseline	Nelfinavir Only	Nelfinavir + 1st Dose Immunotherapy	Hypofractionated Radiotherapy	Post Hypofractionated Radiotherapy	Final Dose of Nelfinavir	Follow-up #1	Follow-up #2
		11-12 weeks of Nelfinavir			Immunotherapy every 2-3 weeks until progression		1-Month Post NFV (+/-) 2 Weeks	6-Months from Enrollment (+/-) 2 Weeks
Tests and Observations								
Physical Exam; ECOG	x		x	x	x	x	x	x
Adverse Events[g]		x	x	x	x	x	x	x
Drug Diary[g]		x	x	x	x	x		
CT Scan[a]	x						x	x
irRECIST 1.1[a, g]	x						x	x
MRI Brain[b]	x							
Biopsy[c, g]							x	x
Laboratory								
CBC w/diff, Hgb, Platelets[d]	x	x	x		x			
CMP[d]	x							
Pregnancy Test[e]	x							
Research Blood Collection[g,h]	x	x	x		x		x	x
Treatment								
Nelfinavir[g]		x	x	x	x	x		
Immunotherapy[i]			x	x	x	x	x[j]	x[j]
3 days x 6-8 Gy[f]				x				

KEY

a. Baseline within 2 months of study entry. Repeat every 10-12 weeks or as clinically indicated

b. Within 2 months of study entry

c. Optional biopsy at end of service

d. Within 30 days of study entry

e. For WOCBP; Within 7 days of study entry

f. A CT simulation will be scheduled to plan for 3 fractions of 6-8gy each to a single lesion. Radiation will commence between 1st and 3rd dose of immunotherapy.

g. Research Procedure

h. 8 yellow top BD #364606 + 1 red top # BD 367820 (Loue King specimen processing)

i. Pembrolizumab, Nivolumab, or Atezolizmab. Continue until progression.