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ALK Digital Pathology outcome prediction Study Protocol

Multi-center
Investigator-initiated
Academic
Retrospective study

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Abbreviations

ALK	Anaplastic lymphoma kinase
BMI	Body Mass Index
CI	Confidence Interval
CNN	Convolutional Neural Network CNN
EMT	Epithelial-to-mesenchymal transition
FISH	Fluorescence in situ hybridization
ERK	Extracellular signal-regulated kinase
FFPE	Formalin-Fixed, Paraffin-Embedded
H&E	Hematoxylin and Eosin
IHC	Immunohistochemistry
mTOR	mammalian target of rapamycin
MIL	multiple instance learning
NGS	Next-generation sequencing
NSCLC	Non-small-cell lung cancer
NR	non-responder
SCLC	Small-cell lung cancer
SMC	Sheba Medical Center
VEGFR	Vascular endothelial growth factor receptor
WSI	whole slide image

1. Background

About 4% of NSCLC patients harbor a rearrangement of the ALK gene, producing a novel chimeric kinase that drives cancer evolvement and progression. ALK inhibitors have been developed and are currently the most active agents for the treatment of such patients, with a response rate around 80% (82.9% for alectinib-treated patients on the ALEX study[1, 2]. Regarding long-term outcome, on the ALEX trial at five-years follow up, the median survival was not reached yet with 62.5% of the alectinib-treated patients still alive [2]. However, a subgroup of these patients do not respond to this treatment or demonstrate only a short-term response followed by rapid progression. Specifically, in the ALEX study, the one-year event-free survival was 68.4% (95% CI 61.0% to 75.9%), meaning 31.6% had progressed or died on alectinib within a year. In the crown study, 78% (95% confidence interval [CI], 70 to 84) were alive and without disease progression at 12 months in the lorlatinib group[3]; thus, with this treatment only 22% had progressed or died within the first year. These results are numerically better with lorlatinib vs alectinib. However, the confidence intervals overlap and in general cross-trial comparisons are not valid. Therefore, and especially considering the very good long-term survival data for alectinib, lorlatinib is considered more relevant for later-line treatments.

No tools are currently available to predict the response to ALK inhibitors and to identify the sub-group of patients that will progress rapidly. Mechanisms of resistance are multiple, consisting of pathway-dependent ALK mutations[4] or amplifications[5], or off-target activation of bypass growth-factor signaling pathways [6]. Additional mechanisms of resistance include epithelial-to-mesenchymal transition (EMT) [7], SCLC transformation, activation of drug-efflux mechanisms; in a large proportion of cases, the mechanism of resistance remains elusive.

Prediction of resistance is limited currently. Variant 3a is recognized as one with higher rate of resistance development, mostly by ALK mutations [8]. In general, resistance to advanced-generation ALK inhibitors is more commonly accompanied by ALK mutations [4,6]. Importantly, identification of ALK fusion by IHC may be a less reliable method than FISH or NGS.[9]

Considering the poor outcome of some of ALK positive patients on ALK inhibitor treatment, it can be speculated that prediction of poor response to such treatment may allow personalized tailoring of more aggressive therapeutic options that are currently being tested in clinical trials. Such options include combination strategies with chemotherapy (e.g. study NCT05200481), combination with VEGF-signaling inhibition (e.g. NCT04227028)[10], or with mTOR or ERK pathway inhibition[11].

Artificial intelligence-based methods of picture analysis have recently emerged as a novel way to characterize tumor specimens. The advent of digital pathology in combination with machine learning and other AI tools appears as the next revolution in the field of pathology[12]. AI tools are currently being tested for diagnosis and quantification of specific tumor characteristics such as IHC stains [13,14] or prediction of specific genetic aberrations[15]. Furthermore, AI allows integration of spatial information about location of specific cells as well as other features not captured by pathologist review. Recent studies including from our center[16], demonstrated the feasibility of prediction of the response to immunotherapy based on machine learning of digital pathology H&E sections of melanoma [17] and lung cancer[16,18]. We aim to use this technique in order identify a sub-group of ALK positive patients that might be candidates for more aggressive treatment options.

2. Objectives

Hypothesis:

Identification of ALK-positive patients' subgroup at risk of rapid progression is possible at the time of diagnosis based on analysis of the H&E (Hematoxylin and Eosin) stained images of tumor sections.

Goal of the study: to develop an AI-driven pathologic image analysis-based classifier that can identify patients unlikely to significantly benefit from the currently utilized first-line ALK inhibitors (advanced-generation ALK inhibitors). Our goal is a classifier with final ROC-AUC value of 0.75.

Endpoint of the study: the development of a classifier of outcome of ALK patients, to identify patients with a high likelihood of progression on 1st-line ALK inh (2nd or 3rd generation inhibitors) within a year.

3. Design

This is a retrospective study.

All data have been collected at different time points during the patients' routine visits at the hospital.

1) Collection of a retrospective set of ALK positive patients with advanced NSCLC that have received an advanced-generation ALK inhibitor treatment as the first ALK inhibitor (i.e. alectinib, lorlatinib, brigatinib or ceritinib): collection of the clinical data, pathologic data, response to treatment and scans H&E images
2) Image analysis of the scanned H&E images, development of a classifier of the data to identify responders vs. non-responders.

4. Origin of the data/biological material

Secondary use and analysis of data.

Potential centers that will participate in the study include any Oncology center that can provide H&E images of diagnostic specimens of ALK+ NSCLC patients with clinical follow up of at least a year. It is expected that each center will be able to contribute 10-20 cases, thus to reach a sample size of 200, about 15 centers are required.

Ethics: Each participating center is independent and will receive approval of the local IRB.

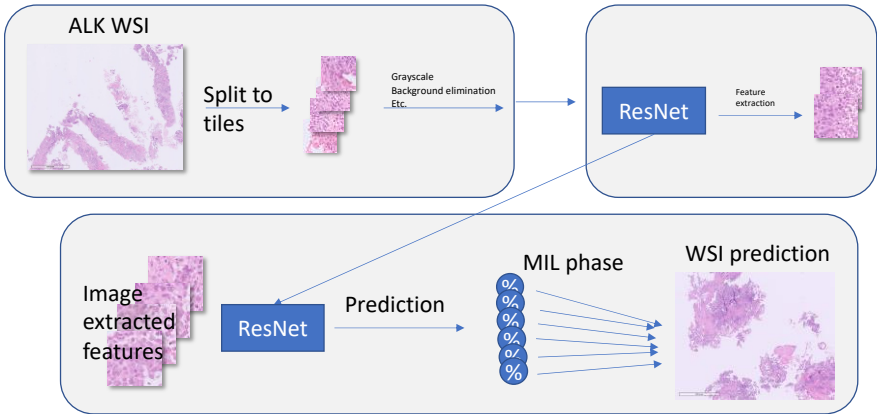
Patient data confidentiality: No identifying information regarding participating patients will leave each of the centers. Participants will be given a study code, the key connecting the code to the patients' identity will be kept at each participating site secured as per local regulations of each site.

5. Study conduct

1) Participating centers will secure an ethics and administrative approval for retrospective collection of clinical data, and provision of a single H&E stained FFPE section. ALK positive patients will be identified from the working database of each center, data will be collected locally, deidentified and collected centrally. Data collected will include demographics (age, sex, smoking status, co-morbidities, BMI), tumor characteristics (histology, ALK testing utilized, specific test results, co-mutations if known, ALK fusion partner and variants, brain metastasis) and treatment (ALK inhibitor utilized, date initiated, dose reductions, treatment breaks, date of documented progression, date of death or last follow-up). We will aim to include only or mostly patients with NGS results available, to allow for robust and valid identification of the ALK-fusion, as well as the variant type and co-mutations, all proven potential confounders. Next, the required H&E slides will be stripped from identifying details, coded. The scanning of pathology slides will be performed; alternatively, the option of sending the image files instead of the slides themselves is available.
2) Image analysis and AI development will be carried out at the Sheba Medical Center, in-house development. The clinical data will be analyzed, tagging study samples as belonging to a responder (R), vs. a non-responder (NR). For the purpose of this study, a NR will be defined as a patient that has progressed or died on an ALK inhibitor treatment within the first year of treatment. Considering most patients would be on alectinib as first-line ALK inhibitor, we expect about 32% of the samples to belong to the NR group. A 30-

70 to 40-60 split between two classes is a reasonable split for the development of a classifier.

The study cases will be randomly split to three: a training cohort, a validation cohort and a test cohort. The cohorts will be stratified by the response to treatment (i.e. equal proportion of R vs. NR cases in each cohort). Next, scanned images will be processed and analyzed. Slides analysis would be done using python using the pytorch packages. Further statistical analysis will be done with R statistical programming.
At first the whole slide image (WSI) is divided into thousands of tiles. These are examined by a convolutional neural network (CNN) to extract tile level features. We will be using Resnet, a common deep learning model used for computer vision as the CNN.[19]The CNN will be trained with multiple instance learning (MIL) at the tile level and later the predicted scores will be aggregated for the WSI level[20]. The final model will be conducted on the slides, to distinguish between R vs. NR. The classifier will be developed on the training cohort, modified if required following processing of the validation cohort and finally tested for efficacy on the test cohort. Cross-Validations techniques will also be used.



6. Inclusion criteria

- Patient aged ≥ 18 years;
- Patient with an oncologic disease;
- ALK positive patients with advanced NSCLC that have received an advanced-generation ALK inhibitor treatment as the first ALK inhibitor (i.e. alectinib, lorlatinib, brigatinib or ceritinib).
- Clinical follow up of at least one year from initiation of ALK inhibitor (unless disease has progressed prior to this date).

7. Exclusion criteria

- Absence of information on the oncologic treatment received;
- Lack of general or specific consent for this study

8. Scientific methods and sample size

The statistical method to define a required sample size for an AI-based algorithm development is not clearly defined at this point. Our target sample size (n=200) is based on several approaches of calculations. Firstly, based on our experience with similar type of studies. For example, an AI algorithm predicting of the response to immunotherapy based on digital pathology was developed based on a set of 76 cases in total [16]. An alternative approach is based on our goal of a classifier with final ROC-AUC value of 0.75 or above with the null hypothesis value to be 0.5. We are aiming for a type 1 error value of 0.05 and for a power of 90%. Approximately 20% of patients are fast progressors (PD within first year of treatment). Total sample size required based on these assumptions is 96 with 16 fast progressors and 80 responders (calculated by MedCalc). Considering our goal to produce a highly robust classifier, and considering the relative lack of

robust manners to calculate sample size, we'll aim for a sample size that is roughly 2-3 times larger than the mentioned numbers, and have set therefore our goal as n=200.
AI-based analysis of images of the training cohort will be conducted using various methods, including machine-learning and deep learning methods. The methods deemed to provide optimal separation between R and NR will be utilized further and validated on the validation cohort and lastly on the test cohort. Training/validation/test sets will be derived from the entire study samples and split about 70:15:15.
Classification of the cases to R and NR in each phase of the study, on each of the three cohorts (training, validation and test) will be examined for dependence on potential confounders by multivariate analysis. Covariates to be considered include: age, sex, BMI, smoking status, time from diagnosis of advanced disease to ALK inhibitor initiation, specific ALK inhibitor used, ALK-fusion of variant 3 or other, co-mutations, histology, method of ALK-fusion analysis, presence of brain metastasis, site of tissue analysis and site of section scanning. Each of these additional clinical/molecular parameters will be utilized if available for more than 70% of the cohort.
In addition, technical parameters such as precise fixation technique, width of sections, type of scanner and similar factors may have significant influence of the image analysis, and care will be taken not to integrate such factors into the algorithm. The generation of the classification algorithm will include at the learning phase repeated shuffling of the cases from various centers and different technical features, thus preventing biased classifications.

9. References

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