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Direct pharmacological assessment of clinically acquired models as a strategy to overcome resistance to tyrosine kinase inhibitors

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We have performed a study using cell lines established from biopsies of clinically resistant non-small cell lung cancers with the aim of discovering therapeutic strategies to overcome acquired resistance. Our results indicate that pharmacological assessment of tumor material might efficiently complement genetic profiling in the future path toward personalized medicine.

Targeted therapies against *EGFR* (epidermal growth factor receptor) and *ALK* (anaplastic lymphoma kinase) mutant lung cancers have had a strong impact on patient health in the last few years.[1,2] Unfortunately, drug resistance almost invariably emerges within a year. Genetic analyses of resistant tumors have led to the identification of a number of resistance mechanisms, in particular mutation of the target itself in over 30% of *EGFR* mutant cancers.[3] In many cases, however, genetic data do not clearly point to alternative therapeutic strategies. Genetic screens[4,5] or detailed characterization of cell lines rendered resistant in the laboratory[6] have also yielded important insights by identifying a number of candidate resistance events. However, it is often challenging to evaluate the relevance of these results to clinical acquired resistance and it is difficult to match the limited number of drugs to the identified resistance mechanisms.

To overcome some of these difficulties and accelerate the discovery of therapeutic strategies to counter drug resistance we have established a pharmacological testing platform that uses clinical biopsies from patients presenting with resistance to tyrosine kinase inhibitors (TKIs) (Crystal et al. Science 2014).[7] Recently described reprogramming culture conditions[8] were used to culture tumor cells from pleural effusion and core biopsies. The resultant cell lines were subjected to next-generation sequencing and a combinatorial drug screen (**Fig. 1**). A total of 55 new cell lines, 20 derived from resistant tumors and 35 derived *in vitro* by chronic drug exposure, were studied with the aim of capturing a large breadth of resistance events and get a sense of the range of mechanisms at play. Instead of using genetic and mechanistic studies to identify drugs that might be efficient in these models we directly tested the ability of drugs to suppress viability of the resistant cells when combined with the original tyrosine kinase inhibitor. We successfully identified sensitizing drugs in the majority of cases and observed high *in vivo* efficacy in all 5 cases tested. Follow-up mechanistic studies confirmed specific targets in different models. Overall, genetic testing was concordant with the drug screen results; however, in a number of cases sequencing did not clearly point to any therapeutic strategy even though sensitizing drugs were identified.

Assessing drug combinations is challenging because of the large number of tests that have to be run. Thus, combinatorial screening cannot be conducted in complex *in vivo* models, let alone in human trials. Cell lines or *in vitro* short-term cultures are essentially the only type of tumor model amenable to broad drug screening. In the study discussed here we aimed to discover drugs that are able to resensitize resistant models to the original tyrosine kinase inhibitor. We therefore used a streamlined combination screen in which 76 drugs chosen for their clinical relevance and ability to target a variety of potential resistance events were tested as single agents or in combination with the original TKI (rather than a 76 by 76 matrix of combinations). Interestingly, although one or more sensitizing drugs could be identified for most resistant models, these drugs were not particularly effective on their own. Thus, the resistant cells had not become hypersensitive to a new drug, and in some cases we could even test this directly because we had matched sensitive and resistant lines. This

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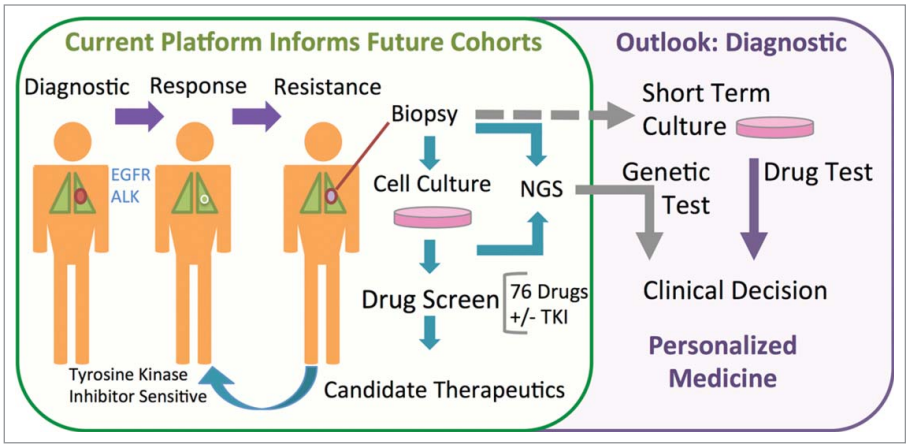

Figure 1. Current studies on the resistance of human tumors to tyrosine kinase inhibitors and future developments. Experimental workflow of the current research platform and future diagnostic applications that integrate genetic analysis and pharmacological assessment of patient derived material. ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; NGS, next-generation sequencing; TKI, tyrosine kinase inhibitor.

supports the idea that in many instances of acquired resistance the original oncogene still drives key proliferation and survival signals.[9]

Genetic analysis of resistant tumors can be very powerful by revealing the presence of previously validated or mechanistically sound resistant events. However, in some instances genetic analysis might yield ambiguous conclusions. For example, multiple candidate events might be identified in one sample with no clear indication of which causes the resistance. This is complicated by the fact that in many cases only the cancer sequence, but not the germline sequence, is available when characterizing the tumor material of patients. Rare germline variants can be flagged as potential drivers of resistance. In our study, sequencing of a given patient's tumor led to the identification of mutations in *MAP2K1* (best known as *MEK1*) and *JAK3* (Janus Kinase 3), both of which were previously characterized as activating mutations and thus candidate drivers of resistance. However, inhibition of MEK1, but not JAK3, sensitized the cells to the original TKI. As it turned out, the *JAK3* mutation was a rare germline variant that was identified as such at autopsy. Importantly, germline variants could participate in the acquisition of resistance and pharmacological assessment might help to identify such cases.

A key finding of our study is that some of the pharmacological screen results were not predicted by sequencing of the resistant tumor samples. Our pharmacological screen identified drugs that, in combination with the original TKI, efficiently suppressed tumor cell viability *in vitro* and *in vivo* although next-generation sequencing did not reveal any mutation in the pathways targeted by these drugs. In particular, a number of the models driven by *ALK* translocation were strongly sensitive to the combination of inhibition of SRC (the tyrosine kinase p60c-src) and inhibition of ALK, but we did not observe mutations in *SRC* or in genes involved in SRC regulation in these models. Indeed, our results suggest that SRC is activated dynamically in response to ALK suppression and that this happens broadly, even in the context of secondary mutation of the ALK kinase domain that renders the initial therapy ineffective (models that were resistant to the first-generation ALK inhibitors were screened using a second-generation inhibitor). Non-genetic events might represent an important component of the acquisition of resistance;[10] in some cases they might be sufficient for clinically manifested resistance whereas in other cases subsequent genetic events might lead to full-blown resistance. In this context, pharmacologic assessment could reveal important non-genetic events that could be targeted in order to block or substantially delay the establishment of resistance.

The aim of the present study was not to directly inform the care of the patients from which the biopsies were obtained but to discover novel therapeutic strategies that could benefit future patients. Encouraged by these results we are currently adapting our platform to develop pharmacological testing as a complement to genetic testing in the path toward truly personalized medicine.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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