Functional Mapping of Multiple Myeloma Kinome Using a Small Molecule Inhibitor Library

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Introduction

In multiple myeloma (MM) and other neoplasias, several kinases have been extensively evaluated as potential therapeutic targets using RNAi-based approaches or pharmacological inhibitors. Attempts to map the functional dependence of MM cells on individual kinases have primarily utilized RNAi, a mechanistic approach inherently dissimilar to small molecule inhibitors that are primarely applied in the clinic. For many of these oncogenic kinases, large numbers of such inhibitors have been designed: these inhibitors often exhibit very similar effect on their primary designated target(s), but also perturb other secondary kinases, which may vary for different inhibitors within the same class. Using large sets of such inhibitors can enable comparative analyses to reveal the functional roles of both the respective primary target(s), as well as non-overlapping secondary targets.

Key technical features of functional kinome mapping

We pursued the functional mapping of the kinome dependencies of 16 MM cell lines, using a panel of 273 kinase inhibitors (Kinase Inhibitor Library L1200, Selleckchem) at 100nM concentration (24-72 h exposure), which target a total of 43 known primary oncogenic targets. The MM cells were previously transfected with luciferase-expressing lentiviral vectors and selected using puramycin. For the assay the cells were plated on 384-well plates (2000-4000 cells/well) and the inhibitors were added by pin transfer one day after. Luciferin was added to the plates on the next day and the cells were incubated for 1h at 37^{O}C. Consecutive measurements of luminescence signal were performed at 24, 48 and 72h of exposure to the inhibitors (Time-Lapse Bioluminescence method, ASH 2014 Abstract #276)

Overview of functional mapping results

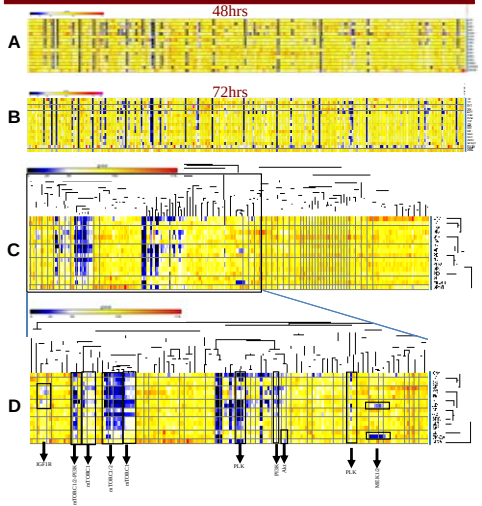

Fig.1 (A,B) Heatmaps summarizing the in vitro response of human MM cell lines to kinase inhibitors of our library after 48hrs (panel A) or 72hrs (panel B) of treatment with these agents. MM cell lines and kinase inhibitors (100nM, 72hrs exposure) are represented in rows and columns, respectively. The color-coded scale denotes the % of viable tumor cell numbers for each experimental condition compared to DMSO control. **(C,D)** Clustering of cell lines base on responses to kinase inhibitors at 48h and magnified snapshot of the area with notably active compounds (black or dark blue cells) against the majority of MM lines; other inhibitors have pronounced activity only against few select MM lines; while most kinase inhibitors did not exhibit substantial anti-MM activity (depicted in yellow). Interestingly, several compounds (e.g. BRAF inhibitors) triggered increase in the number of viable tumor cells (as depicted by red or pink) for at least some MM cell lines of our panel.

Classes of kinases with distinct functional characteristics

•In this study, we observed universally potent activity of inhibitors against:
- •**Aurora** (n=18 compounds), **PLK** (n=5), and
- •**mTORC1/2** (n=20);

•The observations for these 3 classes of kinase inhibitors are consistent with the high proliferative rate of MM cell lines in vitro.

•In contrast, we observed modest to minimal cell-autonomous susceptibility of MM cells to selective inhibitors of PDK1, PI3K (excluding those which also inhibit mTOR), and Akt:

•This latter set of observations suggests that PDK1- and Akt-independent mechanisms mediate the effect of PI3K signaling on the survival of most of these cell lines.

•In addition, we observed lack of response in virtually all tested cell lines to inhibitors of

c-met (n=17 inhibitors)	ALK (n=2)
EGFR superfamily members (EGFR, HER2; n=25 inhibitors)	c-kit (n=3) PDGFR (n=5)
VEGFR (n=21)	Flt3 (n=7)
FAK (n=2)	Syk (n=5)
Src (n=5)	BTK

•These results were observed even in those cell lines with detectable transcript(s) of the respective kinases (Fig.2)

•Notable exceptions to this pattern were inhibitors that, in addition to their primary target, also possess activity to other kinases with known roles in MM (e.g. potent activity of FAK or ALK inhibitors that also target IGF1R, such as TAE226 and GSK1838705A, respectively).

•Consistent with prior experience, several FGFR3 inhibitors showed modest activity against FGFR3- expressing cell lines (e.g. KMS11, KMS18, OPM2, KMS34).

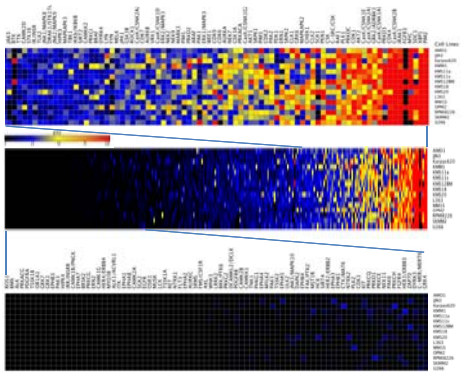

Fig.2 Patterns of expression in human MM cell lines included in our functional mapping for the transcripts of kinases targeted by our kinase inhibitor library

Kinase inhibitors with "non-consensus", heterogeneous, activity across MM cell lines.

•Our screen also revealed several previously underappreciated classes of inhibitors with "non-consensus", heterogeneous, activity across the tested MM cell lines.

•For instance, we identified 3 clusters of cell lines with high (e.g. AMO1, Karpas-620); intermediate (e.g. KMS20, MM1S), and low responsiveness, to 8 different MEK1/2 inhibitors.

• Notably, both Karpas-620 and AMO1 cells are KRAS-mutant, BRAF-wild-type and have inherently high levels of p-ERK; while AMO1 cells also harbor a MEK2-Q60P mutation, previously reported to positively regulate the kinase domain activity of MEK2 and induce resistance of BRAF-V600E mutant melanoma cells to MEK1/2 inhibitors.

•These results raise the possibility that the response to MEK1/2 inhibitors and the role of specific mutations, such as MEK2-Q60P, are tumor-type dependent and/or influenced by concurrent BRAF mutation status.

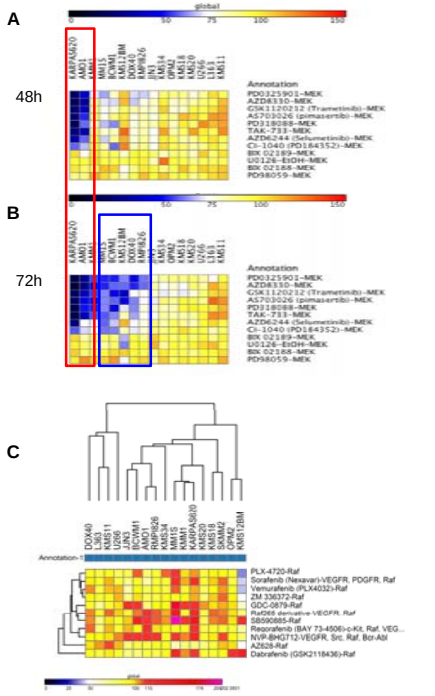

Fig.3 (A,B) Time-lapse bioluminescence assay show early (48hr, panel A) vs. late (72hr, Panel B) responses of MM cell lines to several MEK inhibitors. **(C)** Exposure of several BRAF-widltype MM cell lines to BRAF inhibitors is associated with increase in numbers of viable tumor cells

BRAF inhibitors stimulate proliferation of some MM cell lines

•BRAF inhibitors (n=7) were inactive as cytoreductive agents against our cell line panel of BRAF wild-type cells

•Interestingly, several MM cell lines exhibited significantly increased proliferation upon treatment with these inhibitors.

•This stimulation has been previously noted in melanoma and has been attributed to activation and signaling through C-RAF.

•These observations also suggest that treatment of MM patients harboring both V600E-BRAF mutant and wild-type clones with BRAF inhibitor may decrease the burden of the former clone(s), but select for outgrowth of the latter.

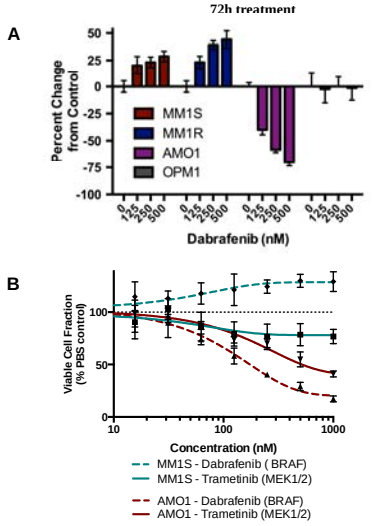

Fig.4 (A) Variation in the responses of MM cell lines to Dabrafenib; 72h exposure. **(B)** Response of MM cell lines to inhibition of BRAF (dabrafenib) and MEK1/2 (trametinib).

Summary of key findings - Translational implications – Future directions

▪Our studies demonstrate the feasibility of phenotypic analyses with panels of tumor cell lines and large libraries of small-molecule kinase inhibitor as an approach to functionally annotate the kinome dependencies in a given neoplasia, such as MM.

▪The use of kinase inhibitors with both overlapping activity against primary targets and distinct effects on ancillary ones, provides insight into the functional role of both primary and ancillary kinases.

▪Insight into possible clinical implications of specific molecular lesions on individualized administration of kinase inhibitors targeting the respective pathway.
- **▪e.g. mutation status of MEK2 or BRAF**

▪We envision that similar approaches can be applied for other types of targets amenable to pharmacological inhibition with small molecule agents

▪Time-lapse CS-BLI provides the basis for kinetic analyses of high throughput evaluation of candidate therapeutics

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