

A WEE1 Pain In the (Head and) Neck

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As with many other types of cancer, patients with head and neck squamous cell carcinoma (HNSCC) that have not responded to surgery, radiation or chemotherapy, have limited treatment options after the disease has spread. A Fred Hutch study published in the journal *Clinical Cancer Research*, led by Russell Moser and Dr. Chang Xu, in the Kemp (Human Biology and Public Health Sciences Divisions) and Méndez (Clinical Research Division) labs, respectively, with significant contribution by the lab of Dr. Carla Grandori (Human Biology Division), sought to identify new therapeutic targets for this disease.

The tumor suppressor p53 is the most commonly mutated gene in head and neck cancer. Because mutations in p53 are common in HNSCC and are associated with metastasis, resistance to therapy and poor patient survival, identifying vulnerabilities of p53 mutant HSNCC is of great clinical interest. "Tumors that harbor p53 mutations are more lethal to the patient, yet there are no tailored therapies for these tumors," said Dr. Eduardo Méndez.

To explore such vulnerabilities of HSNCC cells, the investigators performed a short interfering RNA (siRNA) screen using 5 HSNCC cell lines derived either from primary or recurrent/metastatic tumors that targeted the entire complement of human protein kinases (713 in total, collectively referred to as the kinome), and assayed for cell viability. Due to the heterogeneity of human cell lines, the authors also carried out a similar kinome siRNA screen using 5 low-passage, murine squamous cell carcinoma cell lines (MSCC), which were obtained from mice harboring mutations in p53 pathway genes (*Atm*, *Prkdc*, *p19Arf* and *Trp53* itself), that were subjected to chemical carcinogens to induce squamous cell carcinoma (SCC). 38 out of the 508 kinases shared between human and mouse cells were selected for further studies based on several criteria, such as effects of siRNA knockdown on viability of both human and mouse SCC, p53 mutational status and metastatic potential. Kinases for which inhibition impaired viability of human foreskin fibroblasts were excluded, leaving 28 kinases for follow-up studies. Secondary screening of additional HSNCC lines with independent siRNAs using viability and programmed cell death as endpoints confirmed a set of 10 kinases as essential for cancer cell survival.

To corroborate the siRNA data and to identify compounds for in vivo testing, the researchers tested small molecule inhibitors targeting 6 kinases. AZD1775, a small molecule inhibitor of the WEE1

kinase, had the broadest and most potent effect. Since WEE1 is a kinase that regulates the G2/M transition of the cell cycle, the authors hypothesized that p53-deficient SCC cells were particularly dependent on the G2/M checkpoint. To test this, they performed flow cytometry experiments that demonstrated that inhibition of WEE1 by AZD1775 resulted in unscheduled entry into the cell cycle and programmed cell death. Finally, to ascertain the therapeutic potential for WEE1 inhibition in a preclinical model, the investigators carried out a four arm, double blind study using a HSNCC cell line with high metastatic potential, which demonstrated that AZD1775 potentiates the efficacy of cisplatin, the standard chemotherapeutic agent for HSNCC. "We identified a number of potential kinase targets and focused on the G2/M cell cycle regulator WEE1. Inhibition of WEE1 with a small molecule inhibitor AZD1775 was effective against tumors in mice. These studies directly led to a phase I clinical trial in head and neck cancer patients", summarized Dr. Méndez.

Overall, this study demonstrated that a collaborative effort can rapidly drive a basic research project to a clinical trial and demonstrated the feasibility of exploiting vulnerabilities of cancers cells in the clinic. This approach could lead to better therapies not only for head and neck cancers, but also for other, difficult-to-treat cancers.

[Moser R, Xu C, Kao M, Annis J, Lerma LA, Schaupp CM, Gurley KE, Jang IS, Biktasova A, Yarbrough WG, Margolin AA, Grandori C, Kemp CJ, Mendez E.](#) 2014. Functional kinomics identifies candidate therapeutic targets in head and neck cancer. *Clin Cancer Res*, 20(16), 4274-88. PMID: PMC4135446

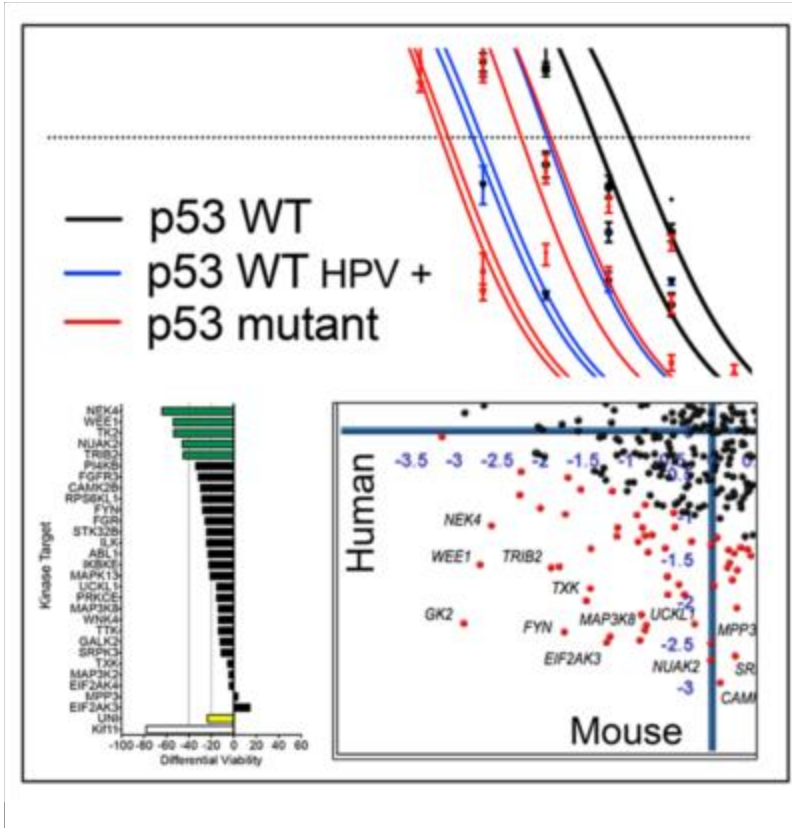


Image provided by Mr. Russell Moser.

Identification of survival kinases in squamous cell carcinoma (SCC) using comparative functional kinomics. Top panel: drug sensitivity of p53 wild-type, p53 wild-type positive for human papilloma virus and p53 mutant SCC cells to chemical inhibition of G2/M kinases. Bottom left: differential cell viability in RNA interference validation screens. Bottom right: Cartesian plot of Z-scores from interspecies kinome identified kinases with highest shared impact on cell viability.