

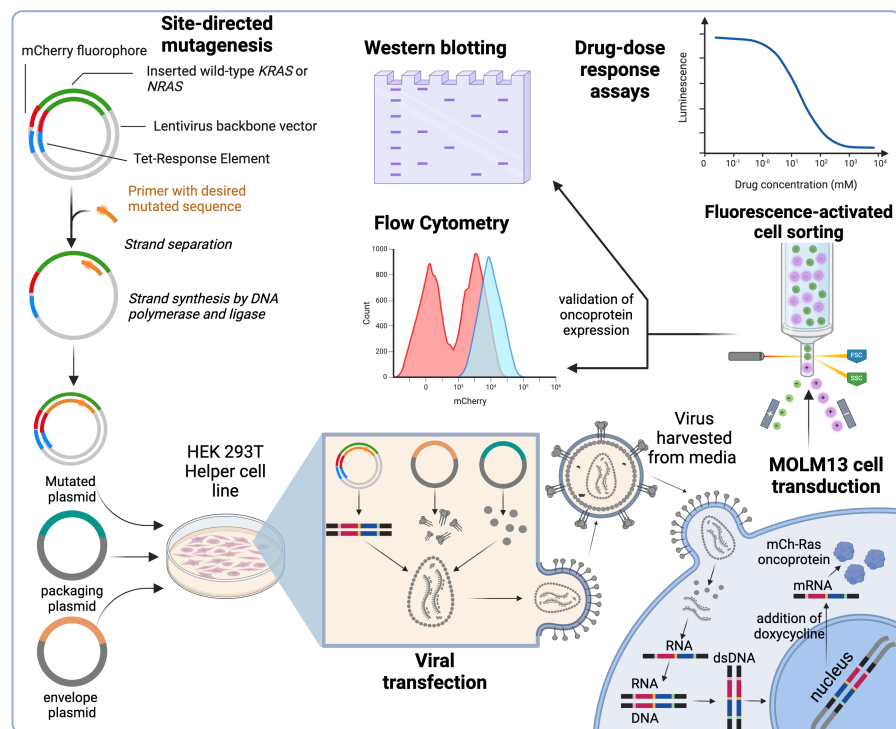
Switchable AML cell model system for investigating chemical inhibitors of oncogenic Ras signaling

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BACKGROUND

Somatic *NRAS* and *KRAS* mutations are overrepresented in pediatric acute myeloid leukemia (AML). The recent development of direct K-Ras^{G12C} inhibitors heralds a potentially effective therapeutic strategy in *RAS*-mutant leukemias, both as a monotherapy and in combination with inhibitors of other Ras/MAPK pathway effectors. To facilitate the investigation of mutation-specific and isoform-selective Ras signaling inhibitors, we set out to generate a cell model system with inducible dependency on common Ras driver mutations.

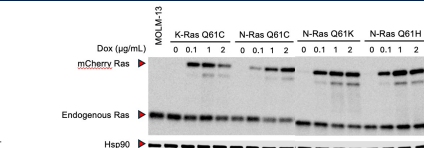
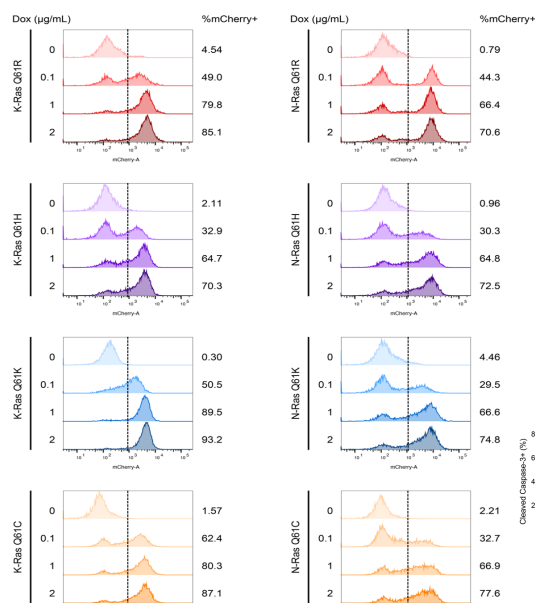
METHODS



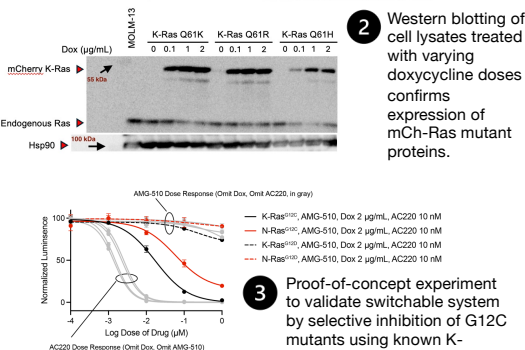
We engineered an inducible cancer cell model system with common *NRAS* and *KRAS* mutations useful for interrogating targeted therapies.

RESULTS

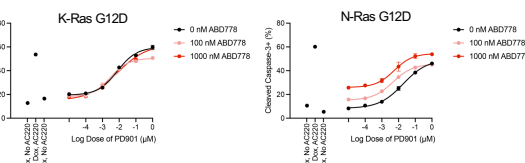
1 Exposure to increasing doses of doxycycline in transduced MOLM-13 cells leads to increasing expression of mCh-Ras mutant proteins by flow cytometry.



2 Western blotting of cell lysates treated with varying doxycycline doses confirms expression of mCh-Ras mutant proteins.



3 Proof-of-concept experiment to validate switchable system by selective inhibition of G12C mutants using known K-Ras^{G12C} inhibitor AMG510.



4 Example of application of cell model system to compare level of apoptosis across Ras isoforms in response to combination therapy using NRAS depalmitoylation inhibitor ABD778 and MAPK pathway inhibitor PD901.

DISCUSSION

The doxycycline dose-dependent expression of Ras oncoproteins demonstrates the successful lentiviral transduction of MOLM-13 cells with oncogenic *RAS* variants. Coupled with co-treatment with the FLT3 inhibitor quizartinib, the engineered switchable MOLM-13 cell model system provides a robust platform for investigating anticancer therapies targeting aberrant Ras/Raf/MAPK signaling. Although our cell model system maintains endogenous expression of wild-type Ras isoforms, the level of induced Ras oncoprotein expression we achieve is sufficient to reproduce the expected response to known Ras pathway inhibitors. For future directions, we will collaborate with other research groups to test new Ras inhibitor candidates, expanding into in vivo efficacy studies.

ACKNOWLEDGEMENTS

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