DCC-2618, a broad-spectrum inhibitor of KIT and PDGFRα mutants, synergizes with inhibitors of the MAPK pathway

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Introduction

Treatment of gastrointestinal stromal tumors (GIST) with the KIT-inhibitor imatinib leads to objective responses or stable disease, but complete responses are rare. Residual signaling through the MAPK pathway via incomplete suppression of KIT, or activation of other pathways that feed into MAPK signaling are possible mechanisms allowing GIST cells to escape death. The status of GIST tumors in response to treatment with KIT inhibitors can lead to rebound tumor growth in the absence of drug or allow for drug-resistance mutations to arise. Inhibitors of the MAPK signaling pathway, such as MEK inhibitors, have been shown to synergize with imatinib to inhibit GIST cell growth in vitro and in vivo (Ran, Cancer Disc. 2015). DCC-2618 is a kinase switch control inhibitor that broadly inhibits the spectrum of primary and drug-resistant mutations in KIT spanning six exons (9, 11, 13, 14, 17 and 18) and in PDGFRA (DCC-2618 is a kinase switch control inhibitor that broadly inhibits the spectrum of primary and drug-resistant mutations in KIT spanning six exons (9, 11, 13, 14, 17 and 18) and in PDGFRA Disc. 2015). Allow for drug-resistance mutations to arise. Inhibitors of the MAPK pathway

DCC-2618 synergizes with MEK inhibitors to induce apoptosis in GIST cells

Figure 1: (A) KIT signals through the MAPK and AKT pathways leading to cell proliferation and survival. MAPK activity leads to greater KIT expression. Reduced MAPK activity leads to a reduction in MAPK and AKT signaling, but residual MAPK signaling or feedbacks from other pathways can allow cells to escape cell death. (B) Combined inhibition of the KIT and MAPK pathways leads to cell death. KIT inhibitors are effective in KIT signaling Synergistic Decisions 6T1 and Supplement GIST Tumor Growth. Cancer Disc. 2015 1A

DCC-2618 and trametinib

Figure 2: DCC-2618 and trametinib combinations induce apoptosis in GIST cell lines. (A) Immuno-blots of pJNK, pERK, Bim and pJAK2 levels in sensitive (GIST-T1) and imatinib-resistant (GIST-T1:D816E and GIST-T1:T670I) cells treated with imatinib (IM, 100 nM) or DCC-2618 (DCC, 100 nM) in the absence or presence of trametinib (Tram, 10 nM) for 48 h. (B) Apoptosis induction in GIST-T1 and GIST-T1:T670I cells as measured by Annexin/V and PI staining. Cells were treated with compounds at indicated concentrations for 24, 48, 72 and 120h and Annexin V and PI.

DCC-2618 and bimetinib

Figure 3: DCC-2618 synergizes with MEK inhibitors to induce caspase activity in GIST cell lines. (A) Caspase activity in GIST cell lines with DCC-2618 and trametinib combination after 24h of treatment. Values represent average of three replicates. (B) Surface mapping of combination dose-response demonstrates synergy in GIST cell lines with DCC-2618 and trametinib. A matrix of various concentrations of drugs was used to measure caspase activity and the BLS5G synergy score was calculated with Combenet software. Caspase 3/7 activity in GIST cell lines with DCC-2618 and bimetinib combination. (C) Surface maps of combination treatments to demonstrate synergy.

DCC-2618 synergizes with MEK inhibitors to induce apoptosis in mastocytosis cells

Figure 4: DCC-2618 synergizes with MEK inhibitors in HMC1.2 cells treated with DCC-2618 alone or in combination with trametinib for 24 h. (B) Surface map of combination treatments with trametinib, cobimetinib and bimetinib demonstrate synergy.

DCC-2618 in combination with MEK inhibitors prevents colony outgrowth of GIST cells

Figure 5: 100 cells for GIST-T1 and GIST-T1-D816E and 500 cells for GIST-T1-T670I were plated in 6 well plates and treated with compounds for 2 weeks. Drugs were washed off and cells were grown in complete media for an additional 10 days. Colonies were stained with crystal violet and counted. (A) Colony outgrowth in GIST cell lines treated with DCC-2618 and trametinib. (B) Average colony counts from three wells. (C) Average colony counts of GIST cell lines treated with a combination of DCC-2618 with bimetinib. Arrows indicate no visually determined colony outgrowth.

DCC-2618/MEK combination induces apoptosis in N-Ras G12D expressing mastocytosis cells

Figure 6: Combination treatment induces colony outgrowth in N-Ras G12D transduced HMC1.2 cells. (A) HMC1.2 empty vector (EV) control and N-rras mutant G12D transduced cells were grown in soft agar for 10 days in the presence of DCC-2618 and trametinib. Drug was washed off and plates were counted for 5 days in complete media. Colonies were stained and photographed. (B) Average colony counts from 3 wells. (C) Caspase activity in EV and N-Ras G12D transduced cells in combination with DCC-2618 and trametinib.

Saturation Mutagenesis

Figure 7: Combination of DCC-2618 and trametinib prevents emergence of drug resistance mediated by non-KIT mechanisms. Ba/F3 cells stably expressing V560D KIT were treated with ENU and grown in the presence of DCC-2618 +/- trametinib for 28 days. Wells exhibiting outgrowth were sequenced for KIT mutations. *V560D (C) indicates that no secondary mutations were found outside of the KIT gene and mutations may have arisen in other pathways, such as the MAPK pathway, that allow for Ba/F3 cell growth. Arrow indicate no outgrowth.

DCC-2618 in combination with trametinib inhibits GIST xenograft tumor growth

Figure 8: DCC-2618 and trametinib treatment results in tumor regressions in a GIST-T1 xenograft model. Mice were treated with DCC-2618 formulated into the diet to achieve ~100 mg/kg/day (red), trametinib (5 mg/kg) (green), or with the combination of drugs (orange). Dosing started on day 10 and ended on day 27. Tumor growth was monitored for additional 40 days. Partial and complete tumor regressions (PR and CR) are shown at Days 27 and 67.

Summary

- KIT inhibitors are cytostatic in GIST cells, and cells start to grow back when drug is removed. The combination of DCC-2618 with a MEK inhibitor induces apoptosis and prevents the outgrowth of colonies even after drug is removed.
- DCC-2618 shows strong synergy in inducing apoptosis in combination with MEK inhibitors trametinib and bimetinib in imatinib-sensitive and, importantly, in imatinib-resistant GIST and mastocytosis cell lines.
- Ras is frequently mutated in advanced forms of systemic mastocytosis and Ras mutant expressing HMC1.2 cells were more sensitive to the combination of DCC-2618 and trametinib.
- In saturation mutagenesis studies, treatment with DCC-2618 prevents the outgrowth of cells with drug-resistant KIT mutations. A few wells did exhibit outgrowth, in which cells presumably had mutations in other signaling proteins or pathways, such as the MAPK pathway. The combination of DCC-2618 with trametinib completely suppressed clonal growth.
- The combination of DCC-2618 and trametinib in a GIST-T1 xenograft model leads to more partial and complete tumor regressions than single agent treatment, even with a short treatment period of 18 days and 40 days of allowing tumors to regrow after dosing was stopped.

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