In life for life the clinic.

To better delay and circumvent resistance now found in novel platform for the evaluation of new drug candidates over mutations, and demonstrate in vivo efficacy further characterized by immunohistochemistry, developed in le. PDX models were mutational and clinical properties in the treatment of gastrointestinal (GIST), patients continue to face a poor practice has vastly improved outcomes for KIT positive stromal tumors (GIST), patients continue to face a poor
drugs. However, as with many kinases, resistance has developed into a clinical dilemma, rendering the drug ineffective. A number of resistance mutations have previously been identified including the well known D816 activating mutation. To overcome this challenge, we have established a set of GIST patient derived xenograft (PDX) models to recapitulate the original patient tumor, including genetics of resistance. In this work, we show four primary GIST samples which were selected based on mutational and clinical profile. PDX models were developed in immunocompromised mice and were further characterized by immunohistochemistry, additional sequencing and pharmacological efficacy. We then evaluated the efficacy of chemotherapeutic agents in these models. We describe imatinib resistance mutations, and demonstrate in vivo efficacy of dasatinib over imatinib in the resistant GIST PDX model. Taken together, this data validates these GIST PDX models as a novel platform for the evaluation of new drug candidates to better delay and circumvent resistance now found in the clinic.

A patient derived xenograft (PDX) platform for development of next generation KIT kinase inhibitors in imatinib-resistant gastrointestinal stromal tumors (GIST)

Introduction

Despite best efforts in the treatment of gastrointestinal stromal tumors (GIST), patients continue to face a poor prognosis. That said, introduction of imatinib into clinical practice has vastly improved outcomes for KIT positive patients. However, as with many kinases, resistance has developed into a clinical dilemma, rendering the drug ineffective. A number of resistance mutations have previously been identified including the well known D816 activating mutation. To overcome this challenge, we have established a set of GIST patient derived xenograft (PDX) models to recapitulate the original patient tumor, including genetics of resistance. In this work, we show four primary GIST samples which were selected based on mutational and clinical profile. PDX models were developed in immunocompromised mice and were further characterized by immunohistochemistry, additional sequencing and pharmacological efficacy. We then evaluated the efficacy of chemotherapeutic agents in these models. We describe imatinib resistance mutations, and demonstrate in vivo efficacy of dasatinib over imatinib in the resistant GIST PDX model. Taken together, this data validates these GIST PDX models as a novel platform for the evaluation of new drug candidates to better delay and circumvent resistance now found in the clinic.

Conclusions

- Molecular Response’s viable tumor bank has been used to establish a large collection of PDX models
- DNA isolated from the tumor collection in conjunction with molecular screening identifies KIT mutant GIST patient tumors
- KIT mutant tumors can be established as PDX models for functional studies to validate resistance/sensitivity mutations
- Exon 11 (delW557K558) exon 17 (Y823D) KIT double mutant preclinically validated in PDX pharmacology studies as patient selection criteria for imatinib-resistance
- KIT defined PDX models now available for future studies of investigational agents

Living Tumor Bank & Patient Derived Xenograft Models

PDX Bank: Molecular Response PDX models have been combined with the Crown Bioscience HuPrime PDX collection now comprising more than 1,600 PDX models across 70 cancer indications. KIT mutant GIST tumors are an example patient type found in the collection.

Targeted PDX Build (KIT mutant GIST)

Sampling of GIST tissue from an aPDX patient for DNA extraction

Patient Samples

- Pre-screening of patient samples in collaboration with Plexxikon, Inc allows for optimal selection of interested gene mutations.
- Secondary screening confirms those mutations, ensuring the genetic stability of the PDX tumors following.

Patient Derived Xenograft Model Development

Patient Derived Xenograft Model Development

Patient Samples: Table depicting clinical characteristics including drug resistance levels, E - extreme, I - intermediate, L - low in Cisplatin (CPLAT), Doxorubicin (DDO), and Etoposide (VP16) analyzed by in vitro chemosensitivity assay. Results from Sanger sequencing identifying mutations across exons 10, 11, 12,13, and 17 in the four patient samples are included.

Patient Derived Xenograft Model Development

Patient Derived Xenograft Model Development

Patient Derived Xenograft Model Development

in vivo Pharmacology (KIT mutation: delW557K558/Y823D)

Patient Derived Xenograft Model Development: (A) Growth curves from four GIST tumor models at passage P1 in GIST 1, GIST 2 and GIST 5 and P0 in GIST 4. (B) H&E images of PDX tissue from mouse at P0 to confirm the histological features of GIST tumors. Retention of histological features in subsequent passages were confirmed by H&E followed by pathology review (data not shown). (C) Mutational analysis depicting the amino acid change to confirm the mutation in KIT is maintained from original patient sample through two passages in the mouse PDX model using ChIP2. Additionally, a mutation in EGFR was gained, while the RB1 mutation was lost. Data was analyzed using Genepool (Stanford, CA).

In Vivo Pharmacology: GIST1 P1 cells were inoculated for each group (n = 10) (A) Median tumor volume (mm³) for vehicle, imatinib, and dasatinib dosing groups with day 0 being stratiﬁcation and day 1 start of dosing. (B) % Body weight change. No drug related toxicity was observed in this study. (C) Individual tumor measurements from each group. (D) Statistical analysis: One-way ANOVA performed on data from day 0 to day 35 for all groups was signiﬁcant; p=0.0002. Tukey’s multiple comparisons test data shown in the table revealed signiﬁcant efﬁcacy of dasatinib in inhibiting tumor growth compared to vehicle as well as compared to the negative control, imatinib. Imatinib was not signiﬁcantly different than the vehicle control.