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Phase I Clinical Trials: Hypothesis Testing

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Developing a pipeline of phase I trials must be accomplished through a series of steps. These include finding a testable hypothesis and delineating certain actions of the drug being tested:

- Does a "drug" act like a drug?
- Does a drug actually accomplish its molecular action/purpose?
- Does it have a clinical effect?
- What accounts for variable effect and loss of effect of this agent?

The completed process is then repeated as necessary to best identify optimal use of any particular agent. Taking this process into account, one can look at the role of BRAF in malignancy. BRAF mutations are found in 7% of cancers and 60% of melanomas.[1,2] The BRAF gene encodes a serum protein kinase, which acts as a signaling molecule in cancer. BRAF has been studied using RNA interference (small interfering RNA or si RNA) in melanoma, and siRNA targeted against BRAF will induce cellular apoptosis.[3] BRAF is also a component in a MAPK (ERK) signaling pathway leading to transcription factor activation that is critical for cell growth, proliferation and survival.[4] Multiple clinical trials have been undertaken to assess BRAF and the use of drugs targeting BRAF to assess the effectiveness, safety, and utility of these agents in patients with melanoma. Such trials involve researching a hypothesis as to how and why a drug may be effective (or ineffective) and what factors surround its beneficial effects or loss of effect once therapy has been initiated.

As an example, sorafenib is an orally-available tyrosine kinase inhibitor (TKI) that targets multiple kinases and also targets tumor progression by inhibiting BRAF. However, its use as monotherapy or in combination with chemotherapy in melanoma has demonstrated limited effectiveness and has not fulfilled the proposed hypothesis of the agent becoming an effective therapy for melanoma. A median progression-free survival (PFS) of 3 months was found in 2 clinical trials with sorafenib as the sole agent, and studies combining the drug with chemotherapy were found to be "dead negative" despite the expectation that sorafenib would target BRAF and slow tumor growth as seen in preclinical studies.[3,5] Sorafenib has been found to affect multiple targets (eg, receptor tyrosine kinases/RTKs, MAPK).[6] In contrast, another agent, PLX4720, was selective for BRAF mutant melanoma both *in vitro* and *in vivo.* This agent was a progenitor for PLX4032 (vemurafenib), which is selective for patients with melanoma who have the BRAF V600 mutation. This mutation has been identified in about 50% of patients with melanoma and has been found to be a biomarker predictive of substantial clinical benefit for treatment of these patients.[7] The optimized formulation of the agent was found to achieve preclinical target exposure to induce tumor regression. Data from 2009 demonstrated that the agent had the best interim overall response in an extension cohort of patients with melanoma, inducing a 70% response rate. Multiple studies at several institutions resulted in remarkable evidence of tumor reduction between baseline positron emission tomography (PET) scans and those performed 15 days post-therapy with vemurafenib. In a study by Sosman, et al, vemurafenib induced a clinical response in over half of patients with previously-treated BRAF V600-mutant metastatic melanoma, with a confirmed overall response rate (ORR) of 53% and median PFS of 6.8 months.[8]

However, resistance to BRAF inhibitor-based therapy has evolved over the past 7 years, and new data suggest improved overall survival with a combination of both BRAF and MEK inhibition vs chemotherapy or BRAF inhibition therapy alone.[9] For example, studies have demonstrated that the combination of a selective BRAF inhibitor, dabrafenib, and a selective MEK inhibitor, trametinib, improved both PFS and OS at 3 years (22% and 44%, respectively). However, the genomic landscape of patients who participated in these trials showed alterations in previously identified recurrently mutated melanoma genes (TP53, PTEN, CDKN2A, CTNNB1) and in MAPK-pathway inhibition resistance genes (MAP2K1, MAP2K2, NF1, MITF). In addition, the CDKN2A mutation and deletion were significantly associated with poorer OS and PFS, with preclinical data

suggesting that combining BRAF and MEK inhibitors with CDK4/6 inhibitors could be a promising future strategy for therapy.[10]

Several mediators of resistance have been implicated in TKI therapy of advanced melanoma. These include the multitude of receptor tyrosine kinases that could be targeted for therapy, PTEN loss, persistent pS6K, persistent formation of the eIF4F complex, the YAP-Hippo pathway, and the NOTCH pathway, which is inversely related to MITF expression. While most drug-sensitive cell lines and tissue biopsies in V-600-mutant melanoma have demonstrated high expression and activity of the melanocytic lineage transcription factor MITF, those intrinsically resistant cell lines and biopsies displayed low MITF expression.[11] Transcriptional heterogeneity has been demonstrated in melanoma metastases, with malignant cells within a single tumor showing heterogeneity associated with cell cycle, spatial context, and a drug resistance program. This may include a shift in the transcriptional state with BRAF/MET inhibition.[12] Potential targets for the MITF low phenotype include the role of the β-catenin pathway, increased oxidative phosphorylation/mitochondrial biogenesis, and increased BCL2A1 expression. For example, the combination of JAK, BRAF, and EGFR inhibitors in melanoma therapy overcomes drug resistance; RNF125 expression in melanoma specimens has been found to be inversely correlated with resistance to BRAF inhibition, with upregulation of JAK1 due to attenuated RNF125 expression underlying this resistance.[13] In addition, MEK inhibition broadly decreases shedding of multiple RTKs, and potentially reduction of RTK shedding may highlight a mechanism for bypass signaling in cancer drug resistance.[14]

Summary:

Clinical trials evaluating therapy in oncology are constantly ongoing, assessing drug effectiveness, safety, and utility. However, many avenues and pathways for drug resistance complicate application of these therapies, and multiple hypotheses and targets must be assessed and evaluated (and often reevaluated) to not only find the best targets for therapy, but to assess agents in combination and to identify barriers to treatment and mechanisms of resistance. Starting with the original (and failed) hypothesis that sorafenib should be effective in treating melanoma, multiple hypotheses have been explored to better understand the genomics behind the disease, the mechanisms and drug action and resistance, and the best ways to combine agents for therapy or address particular tumor characteristics to optimize use of particular drugs or drug combinations to optimize therapy and improve patient outcomes. As demonstrated with the treatment of melanoma, multiple hypotheses must be tested and either repeated or discarded to better identify therapy targets and potential agents for treatment and advancing disease management.

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