Challenges in combining novel molecularly targeted agents in cancer medicine

Combining novel molecularly targeted agents is probably one of the most critical and complex challenges in cancer medicine today [1]. Although we currently have a burgeoning armamentarium of targeted agents in early phase clinical trials, it is clear that when administered as single agents, patient benefit is limited by the inevitable development of drug resistance. There are multiple reasons for this, including the disruption of signaling feedback loops and development of crosstalk and compensatory mechanisms of resistance. Recent studies have highlighted issues of intra- and intertumoral heterogeneity, indicating that monotherapy-targeted approaches are unlikely to result in durable antitumor efficacy [2, 3]. In light of this, recent drug development efforts have focused on combining novel targeted agents, with the primary aims of circumventing early drug resistance mechanisms and prolonging patient benefit [4].

The importance of such combinatorial approaches is reflected in the release of recommendations from the US Food and Drug Administration (FDA) on the co-development of investigational agents [5]. Several criteria have been proposed for the selection of individual drugs for such combination strategies. These include the existence of compelling biological evidence for the use of the combination, data that the novel agents cannot be developed individually, and preclinical studies showing that the combination not only has substantial activity, but is also associated with greater and more durable antitumor response or a better toxicity profile than the individual agents.

In the article by Tolcher et al. [6], the investigators have undertaken a phase IB/II trial involving the combination of the mitogen-activated protein kinase kinase (MEK) inhibitor trametinib and mammalian target of rapamycin (mTOR) inhibitor everolimus. Both drugs are approved targeted therapies that have been shown to benefit patients as single agents in multiple cancers, including BRAF V600-mutation-positive melanoma [7] and renal cell carcinoma [8], respectively. This rational combination involves the horizontal blockade of mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinase (PI3K) signaling pathways, which are involved in the regulation of multiple key cellular functions. Importantly, there are strong preclinical data to support the simultaneous blockade of both pathways in cancers that harbor genetic aberrations along these signaling trunks [9].

While this is essentially a negative clinical trial for failing to establish a recommended phase II combination dose and schedule with acceptable tolerability and adequate drug exposure, the study clearly showcases the current challenges associated with combining targeted agents. It also dispels the notion that targeted therapy combinations are associated with minimal normal tissue toxicities, and clearly demonstrates that two great drugs do not always make a good combination. Such observations with targeted therapy combinations are not new, with previous studies involving other approved molecular agents also leading to ‘supra-additive toxicities’ [10, 11]. Although no obvious pharmacokinetic drug interactions were observed with this study combination, there were clear overlapping toxicities with both drugs, including mucosal inflammation, stomatitis, fatigue and diarrhea, which prohibited the achievement of clinically relevant drug exposures. Significantly, treatment-related grade 3 toxicities were observed in 24 out of 67 (36%) patients, with mucosal inflammation and liver function derangement frequently observed, necessitating dose interruptions and reduction. Rash was surprisingly not frequently observed, compared with previously undertaken studies of both drugs as single agents. Relatively high rates of drug-related toxicities have also been observed in other studies involving the combined targeting of other key MAPK and PI3K pathway substrates, such as the inhibition of MEK with PI3K or AKT [12, 13].

The study by Tolcher and co-workers also demonstrates that in such scenarios where the single-agent maximum tolerated dose (MTD) of each targeted therapy component has already been well delineated in previous phase I trials, drug scheduling appears to be more critical than dose finding. Since the MTD range has already been established, starting doses may be selected close to the previously established MTDs, thus reducing the need for multiple dose escalation cohorts. For example in this study, Tolcher and co-workers used 25% of the 2 mg recommended monotherapy trametinib dose used in melanoma treatment, and 50% of the 10 mg recommended single-agent dose of everolimus utilized in renal cell cancers. With regards to drug scheduling, the use of intermittent schedules may improve tolerability and potentially widen the therapeutic window of combinatorial regimens. The pulsatile dosing of drugs will also permit a higher degree, albeit shorter duration, of target and pathway blockade compared with continuous dosing, and may
potentially minimize tumor cell adaptation and eventual secondary drug resistance [1]. In order to guide dose and scheduling in clinical trials, it will be important to define preclinically the extent and duration of target and signaling pathway inhibition required for optimal antitumor benefit and an acceptable therapeutic window.

The study involved a total of 10 different dosing combinations involving 67 patients, a rather large number for a phase I study. We have previously proposed a novel phase I trial design for targeted agent combinations, which compares different drug schedules between single-patient cohorts in parallel from the outset of the study [1]. With such a strategy, intrapatient dose escalation is undertaken in individual patients receiving different schedules of the combination. Dose schedules found to be toxic or have poor pharmacokinetic–pharmacodynamic profiles are discontinued, whereas promising schedules are taken forward through expanded cohorts of patients, preferably involving those with specific molecularly defined tumor types. Bayesian adaptive design models incorporating the analysis of data from multiple doses and schedules may also be considered with such a design. Such an approach has the potential to optimize drug exposures in individual patients, compare multiple combination schedules and reduce the need for a large phase I trial.

As the combination regimen in the study by Tolcher and co-workers involves two active drugs, the preliminary antitumor responses observed may simply be due to one of the two drugs and ultimately do not prove the value of the combination [1]. In view of the frequent combination-related toxicities observed, and since both trametinib and everolimus are approved for single-agent use, the sequential monotherapy administration of both drugs to patients with molecularly defined tumors probably represents a more feasible and practical approach. Such a treatment strategy should ideally be guided by the molecular characterization of serially obtained tumors or circulating biomarker specimens, such as plasma DNA, for the monitoring of emerging resistant clones [14].

Pharmacodynamic studies could potentially have provided useful mechanistic insights into the levels of target and pathway inhibition achieved with this combination for correlation with treatment-related toxicities and antitumor activity. Although pharmacodynamic studies utilizing paired tumor biopsies may be logistically challenging, normal patient tissue such as platelet-rich plasma [15] and hair follicles [16] are emerging as promising surrogates, which may be obtained serially before and during treatment. It is interesting but probably not surprising that in contrast to the horizontal blockade of critical nodes along different pathways, the vertical blockade of multiple points along a single signaling trunk has led to great success. For example inhibiting MEK and BRAF has led to patient benefit with good tolerability in BRAF V600-mutation-positive melanoma [17, 18]. It is likely that the synergistic inhibition of a single signaling pathway may result in a wider therapeutic window, in contrast to the blockade of multiple critical cellular functions with the horizontal inhibition of different pathways.

As demonstrated through Systems Biology models, it is now insufficient to simply assume that a signaling pathway is inhibited when a specific drug is administered [19]. Instead, it will be important to understand and quantify the degree of influence of each individual pathway to the combination regimen in different molecularly driven preclinical tumor models. This is especially vital since combination-related toxicity and efficacy profiles are likely to be dependent on the level of pathway blockade achieved along each signaling trunk, because of the disruption of distinct feedback loops or crosstalk development at different points along both pathways. In the future, it will be interesting to investigate the feasibility of combining a catalytic mTOR complex-1/2 (mTORC1/2) inhibitor with trametinib, instead of an allosteric mTOR inhibitor. Such ATP-competitive mTORC1/2 inhibitors have been shown to be able to circumvent intrinsic and acquired resistance associated with rapalogs due to AKT activation following inhibition of the S6 kinase and insulin receptor substrate-1 feedback loop [20].

To conclude, the development of targeted drug combinations is now commonplace and is associated with numerous challenges [1]. As shown in the study by Tolcher and co-workers, drug-related toxicities may ultimately limit the further development of rational combinations. To address such issues in the future, the development of targeted therapy combinations will require novel hypothesis-testing, biomarker-driven clinical trial designs to optimize both drug dosing and scheduling, and ideally be supported by emerging computational and experimental network biology science [4].

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Pushing the boundaries of somatic copy-number variation detection: advances and challenges

At the 2014 Annual Meeting of the American Society of Human Genetics in San Diego, thousands of attendees crammed into a large, overcrowded auditorium to listen to the invited session on copy-number variation (CNV). There, thought leaders such as Mark Gerstein and Steve McCarroll delivered talks on approaches to detect and interpret novel CNVs. One big takeaway point from that morning: new technologies and computational methods are two main driving forces behind recent advancements. On one hand, emerging technologies such as long-read sequencing [1], single-cell sequencing [2], droplet digital PCR [3], and nano-channel arrays [4] generate much excitement. On the other, refinement of computational techniques to normalize sequencing data and detect CNV at greater resolution allows for large-scale CNV studies, especially as more sequencing data are being generated and becomes available. In this issue of *Annals of Oncology*, Favero et al. [5] take the CNV calling algorithm one step further. Their method, Sequenza, not only improves the detection accuracy of sample purity, tumor ploidy, and absolute CNV calls, but also provides allele-specific copy-number estimates. This will allow for more comprehensive understanding of the functional impact of copy-number alteration.

In the course of cancer evolution, normal cells acquire and accumulate aberrations in their genomes. Among the types of genetic aberrations, CNVs affect the largest fraction of the genome [6, 7], with events ranging in size from a single exon to the whole genome [8]. CNVs are signatures of genome instability and deregulation, and duplication of oncogenes has been shown to cause several types of cancer [6]. In a recent ‘pan-cancer’ survey of CNVs, whole-genome doubling is observed in 37% of cancers and is correlated with other events such as *TP53* mutations and *CCNE1* alterations [9].

Next-generation sequencing, especially exome sequencing, has become an indispensable tool in the study of cancer biology. Detection of CNVs from sequencing data is enabled by computational approaches that normalize read counts and identify CNV regions. Several methods have been published over the past few years. Largely they can be characterized by the intended application (germline, somatic), sequencing technology (whole genome, whole exome, targeted), type of samples (one genome, several genomes, paired tumor-normal), and detection strategy (read depth, paired-end, split read, assembly) (for review, see [10] and [11]).

In the context of cancer research, methods that use tumor-normal paired samples are most suitable for detecting somatic CNVs because (i) they exclude germline CNVs from consideration and (ii) the comparison of reads from the same region reduces positional biases such as GC content and uniqueness of the sequences. Generally, these methods proceed by taking the ratio of normalized read counts between tumor and normal samples in a given window. In theory, the ratio of 1 represents no copy-number change; the ratio of 1.5 presents one copy gain; and the ratio of 0.5 represents one copy loss (assuming diploid genome). However, because most tumor biopsy samples are mixtures of normal and cancer cells, the read ratios tend to deviate from the expected values of 0.5 or 1.5 toward 1 (the ‘null’), thus reducing the power of detection. Taking into