Developing Drugs for Tissue-Agnostic Indications: A Paradigm Shift in Leveraging Cancer Biology for Precision Medicine

Nathan D. Seligson[1,2,*], Todd C. Knepper[3], Susanne Ragg[4] and Christine M. Walko[3]

Targeted therapies have reshaped the landscape of the development of cancer therapeutics. Recent biomarker-driven, tissue-agnostic clinical trials represent a significant paradigm shift in precision cancer medicine. Despite their growth in preclinical and clinical studies, to date only a few biomarker-driven, tissue-agnostic indications have seen approval by the US Food and Drug Administration (FDA). These approvals include pembrolizumab in microsatellite instability-high or mismatch repair deficient solid tumors, as well as both larotrectinib and entrectinib in *NTRK* fusion-positive tumors. Complex cancer biology, clinical trial design, and identification of resistance mechanisms represent some of the challenges that future tissue-agnostic therapies have to overcome. In this Review, we present a brief history of the development of tissue-agnostic therapies, comparing the similarities in the approval of pembrolizumab, larotrectinib, and entrectinib for tissue-agnostic indications. We also explore the future of tissue-agnostic cancer therapeutics while identifying important challenges for the future that drugs targeting tissue-agnostic indications will face.

Cancers have traditionally been classified and treated based on their pathologic classification and tissue of origin. Advances in the molecular understanding of oncogenesis have allowed for stratification of malignancies into molecularly similar tumors, both within and across tissue of origin, better establishing prognosis and therapeutic treatment. In many cancers, major treatment decisions are made early in the care of these patients based on the molecular characteristics of their cancer.[1,2] With the rapid growth in the understanding of the cancer genome, the drug approval process has progressed from the historical assumption that cancers classified by tumor histology represent a homologous underlying population. Recently, the US Food and Drug Administration (FDA) granted approval for three molecularly targeted therapeutic agents for biomarker-defined diseases, agnostic to tumor histology. This paradigm shift in drug development has driven the field of precision medicine to seriously consider the molecular depths of cancer biology to identify opportunities to improve the treatment of patients with cancer.

Despite the recent successes in tissue-agnostic therapy, mounting evidence suggests that primary drug resistance mechanisms may still depend on histology and the cellular lineage.[3] Here, we review a brief history of the development of tissue-agnostic therapies in order to understand the successful development pathways used to obtain tissue-agnostic approval by the FDA, while considering challenges and opportunities that face the field of precision medicine in regard to tissue-agnostic therapy.

CURRENT TISSUE-AGNOSTIC DRUG APPROVALS
Mismatch repair deficiency and pembrolizumab: Expanding indications

Immune checkpoints, used by T cells to differentiate foreign from host tissue, are commonly hijacked in cancer in an effort to evade the immune system's antitumor activity. Therapeutically inhibiting these checkpoints, therefore, activates an immune-mediated antitumor response.[4] The first immune checkpoint inhibitor therapy (ICI) approved in 2011 by the FDA, ipilimumab, targeted cytotoxic T-lymphocyte–associated protein 4 (CTLA4) for the treatment of patients with unresectable or metastatic melanoma. Ipilimumab was followed by the development of agents targeting programmed cell death protein 1 (PD-1), pembrolizumab and nivolumab, each approved in 2014 for the treatment of advanced melanoma (**Figure 1a**). Over the next few years, pembrolizumab and nivolumab would gain approvals for use in multiple cancer types. Due to demonstrated efficacy in numerous cancers, efforts to establish biomarkers to more reliably predict benefit from ICIs across cancers have been widespread. Cancer mutability has been proposed as a biomarker of ICI response.[5] The biologic connection between cancer mutability and ICI response is based on the premise of missense mutations in tumors leading to the generation of neo-antigens, which serves to enhance recognition by the immune system.

Phenotypic measures of cancer mutability, including DNA mismatch repair (MMR) efficiency, and microsatellite instability (MSI), have been assessed for correlation with lymphocytic tumor infiltration and ICI response.[6,7] A preliminary study
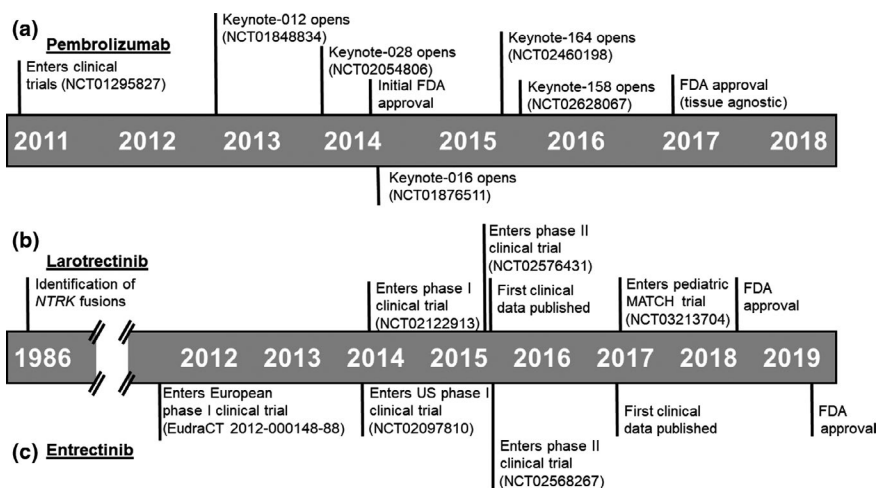
Figure 1 Clinical drug development timeline for FDA-approved tissue-agnostic therapies. Timeline of clinical development for current FDA-approved tissue-agnostic therapies including (**a**) pembrolizumab, (**b**) larotrectinib, and (**c**) entrectinib. FDA, US Food and Drug Administration.

including 11 deficient MMR (dMMR) and 21 proficient MMR colorectal cancer (CRC) patients as well as 9 patients with non-CRC dMMR cancers was used to assess the activity of pembrolizumab based on dMMR status with overall response rates (ORR) reported of 40%, 0%, and 71%, respectively. This proof-of-concept study effectively demonstrated the predictive potential of dMMR across cancer types.[8] This clinical trial was not designed for the FDA's approval process, and ultimate approval was based on polled analysis from five independent clinical trials (Keynote-016, Keynote-164, Keynote-012, Keynote-028, and Keynote-158), each with different eligibility criteria and

dosing regimens. It is noteworthy that this process did not provide a predefined sample size and that 50% of the non-CRCs included in the tissue-agnostic indication application were retrospectively identified from the aforementioned clinical trials. Based on pooled ORR and safety data, pembrolizumab was granted the first tissue-agnostic approval by the FDA for the treatment of adult and pediatric patients with unresectable or metastatic, MSI-High (MSI-H) or dMMR solid tumors who have progressed following prior treatment and who have no satisfactory alterative treatment options.[9] The combined trials included 149 patients and included 15 cancer types (**Table 1**).[10]

Table 1 Clinical response to pembrolizumab in microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancers

Cancer type[11]	n (%)	Overall response rate (% (95% CI))	Duration of response (range, months)
Total	149 (100%)	39.6% (31.7–47.9)	1.6[a]–22.7[a]
Colorectal	90 (60.4%)	36% (26–46%)	1.6[a]–22.7[a]
Noncolorectal	59 (39.6%)	46% (33–59%)	1.9[a]–22.1[a]
Endometrial	14 (9.4%)	36% (13–65%)	4.2[a]–17.3[a]
Biliary	11 (7.4%)	27% (6–61%)	11.6[a]–19.6[a]
Gastric or GE junction	9 (6.0%)	56% (21–86%)	5.8[a]–22.1[a]
Pancreatic	6 (4.0%)	83% (36–100%)	2.6[a]–9.2[a]
Small intestinal	8 (5.4%)	38% (9–76%)	1.9[a]–9.1[a]
Breast	2 (1.3%)	PR, PR	7.6–15.9
Prostate	2 (1.3%)	PR, SD	9.8[a]
Bladder	1 (0.7%)	NE	NA
Esophageal	1 (0.7%)	PR	18.2[a]
Sarcoma	1 (0.7%)	PD	NA
Thyroid	1 (0.7%)	NE	NA
Retroperitoneal adenocarcinoma	1 (0.7%)	PR	7.5[a]
Small cell lung	1 (0.7%)	CR	8.9[a]
Renal cell	1 (0.7%)	PD	NA

CI, confidence interval; CR, complete response; GE, gastroesophageal; NA, not applicable; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease.
[a]Ongoing response.

Pooled analysis reported an ORR of 39.6% (95% CI, 31.7–47.9%), which included a significant portion of durable (78% ≥ 6 months) and complete responses (7.4%). Follow-up studies confirmed the activity of pembrolizumab in MSI-H or dMMR solid cancer, finding that dMMR cancers were sensitive to pembrolizumab regardless of histology.[11] Additionally, based on the populations studied here, the accelerated approval for pembrolizumab in MSI-H or dMMR solid cancers included both pediatric and adult patients, with the exception of pediatric patients with MSI-H central nervous system cancers. Further postmarketing requirements from the FDA specified inclusion of 124 dMMR CRC patients and at least 300 non-CRC patients for a minimum follow-up of 12 months to better characterize response. The FDA also required follow-up study to include ovarian, prostate, non-small lung and thyroid cancers as well as pediatric patients. Notably, in April 2020 the FDA granted priority review for pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors with tissue tumor mutation burden-high status, another tumor-agnostic biomarker defined as ≥ 10 mutations/megabase as measured by the FoundationOne CDx assay.[12]

Interestingly, this initial approval came without a companion diagnostic test. While the majority of patients included in the efficacy analysis were identified using local laboratory-developed, polymerase chain reaction tests for MSI-H status or immunohistochemistry tests for dMMR, the approval also allows for the potential of next-generation sequencing (NGS) assays to identify MSI-H.[9] Several commercial NGS assays now report MSI status alongside other genetic alterations. Development of an FDA-approved companion diagnostic is currently underway.

In the case of pembrolizumab, the pathway to receiving a tissue-agnostic indication began with a tissue-specific indication in melanoma and expanded into other cancer type–specific approvals before correlative and translational studies identified MSI-H/dMMR as a key biomarker of response. This pathway to approval was driven by the extraordinary signal of MSI-H/dMMR–driven cancers, promoting initial hypothesis testing, and extending to tumors with lower prevalence of MSI-H/dMMR–driven cancers.

Targeting NTRK fusions: Designing a drug for a target

Neurotrophic receptor tyrosine kinase (NTRK) genes (NTRK1, NTRK2, and NTRK3) are key regulators of neuronal and embryonic development, which, when activated, trigger downstream proliferative pathways.[13] NTRK fusions were first reported to be oncogenic in 1986 and have now been identified as a unique oncogenic phenotype marked by constituently activated growth.[14] Key to the oncogenic activity of NTRK fusions is the conservation of an intact tropomyosin receptor kinase (TRK) kinase domain, providing potential for therapeutic targetability. Shared kinase structure between NTRK and other kinases, notably ALK and ROS1, provided opportunities for cross-development of TRK inhibitors with drugs already in development.[15] Therefore, development of drugs targeted to NTRK were able to follow established chemical development programs. Early preclinical study of NTRK fusions identified oncogenic activity regardless of cellular histology due to the consistent oncogenic phenotype of NTRK fusions.[16]

Additionally, NTRK fusion–positive cancers had relatively few secondary mutations compared with non-fusion-driven cancers, thus resembling a more molecularly homogenous group.

Larotrectinib, an inhibitor specific to TRK, first entered clinical trials as a dose-finding study that did not require patients' tumors to be positive for the NTRK fusion in early 2014, with the first publication of the preclinical and clinical effect in NTRK fusion–positive cancers coming in 2015 (**Figure 1b**).[17] This publication described the clinical course of a patient achieving a near-complete response to therapy, exceptional for their clinical case. Due to the rarity of NTRK fusions, traditional randomized clinical trials were deemed not feasible to study TRK inhibitors. Because of this, clinical trials designed to include multiple tumor types were used to study the clinical efficacy of early TRK inhibitors in both adult and pediatric patients.

Following preclinical development, regulators worked alongside researchers to design a nonrandomized clinical trial strategy that would be powered to identify a signal of response. In the agreed upon study, the first 55 patients with NTRK fusions treated with larotrectinib would be presented to the FDA for conditional approval. The predetermined efficacy end point of ORR by independent radiology review was reported in a pooled analysis of three clinical trials, NCT02122913, NCT02637687, and NCT02576431, which included patients whose age ranged from 4 months to 76 years who exhibited fusion in any of the three known NTRK genes.[18–20] In this combined study of 55 subjects, 75% (95% CI, 61–85%) achieved an overall response of partial or better (**Table 2**).[21] Across studies, tissue types, and age ranges, clinical response remained consistent. Based on this pooled analysis, the FDA granted accelerated approval contingent on postmarketing requirements, including the characterization of the response in CRC, melanoma, central nervous system, and non-small lung cancers, as well 40 additional tumors that had not yet exhibited a high response rate. To be able to better characterize the duration of the response, patients are required to be followed for a minimum of 12 months. This accelerated approval included adult and pediatric patients with solid tumors exhibiting an NTRK fusion, without a known acquired resistance mutation, which are metastatic or unamenable to surgical resection, and for whom no alternative treatments are available. It is notable that this approval represents the first time that the initial approval for a cancer therapeutic agent was agnostic to tissue type.

Entrectinib has also demonstrated activity against a number of other kinases, including TRK, ROS1, and anaplastic lymphoma kinase (ALK), and was developed as an additional treatment option for NTRK fusion–positive cancers. Pooled analysis of three clinical trials, ALKA, NCT02097810, and NCT02568267, included both pediatric and adult patients who exhibited a fusion in any of the three known NTRK genes.[22] In this combined study of 54 subjects, 57% (95% CI, 43–71%) achieved either complete or partial response to therapy (**Table 2**).[23] Median duration of response was noted to be 10 months (95% CI, 7.1–not estimable) with a median follow-up of only 12.9 months. Similar to larotrectinib, early clinical data for entrectinib in NTRK fusion–positive cancers suggest significant response across tumor types. Entrectinib entered clinical trials in early 2012 and was granted accelerated approval by the FDA based on a pooled analysis of phase I trials (**Figure 1c**).[24] Entrectinib shares a similar tissue-agnostic indication with larotrectinib, with

Table 2 Clinical response to TRK inhibitors in *NTRK* fusion–positive cancers

Cancer type	Larotrectinib[22]			Entrectinib[24]		
	n (%)	Overall response rate (% (95% CI))	Duration of response (range, months)	n (%)	Overall response rate (% (95% CI))	Duration of response (range, months)
Total	55 (100%)	75% (61–85%)	1.6[a]–33.2[a]	54 (100%)	57% (43–71%)	2.8–26.0[a]
Appendix	1 (1.8%)	SD	NA		NA	
Breast	1 (1.8%)	PD	NA	6 (11.1%)	83% (36–100%)	4.2–14.8[b]
Cholangiocarcinoma	2 (3.6%)	SD, NE	NA	1 (1.9%)	PR	9.3
Colorectal	4 (7.3%)	25% (NA)	5.6[b]	4 (7.4%)	25% (NA)	4.8[b]
Gastrointestinal stromal tumor	3 (5.5%)	100% (29–100%)	9.5–17.3[a,b]		NA	
Gynecologic	NA			2 (3.7%)	PR	20.3[b]
Infantile fibrosarcoma	7 (12.7%)	100% (59–100%)	1.4[a]–10.2[a]		NA	
Lung	4 (7.3%)	75% (19–99%)	8.2–20.3[a]	10 (18.5%)	70% (35–93%)	1.9[b]–20.1[b]
Melanoma	4 (7.3%)	50% (NA)	1.9–17.5[a]		NA	
Neuroendocrine	NA			3 (5.6%)	PR	5.6[b]
Pancreas	1 (1.8%)	0% (NA)	NA	3 (5.6%)	PR, PR	7.1–12.9
Salivary gland	12 (21.8%)	83% (52–98%)	7.7–27.9[a]	7 (13.0%)	86% (42–100%)	2.8–16.5[b]
Soft tissue sarcoma	11 (20.0%)	91% (59–100%)	3.6–33.2[a]	13 (24.1%)	46% (19–75%)	2.8–15.1
Thyroid	5 (9.1%)	100% (48–100%)	3.7–27.0[a]	5 (9.3%)	20% (NA)	7.9

CI, confidence interval; NA, not applicable; NE, not evaluable; *NTRK*, neurotrophic receptor tyrosine kinase gene; PD, progressive disease; PR, partial response; SD, stable disease; TRK, tropomyosin receptor kinase.
[a]Ongoing response. [b]Value at data cutoff.

the exception of a restriction for the use of entrectinib in *NTRK* fusion–positive patients to those over 12 years of age.

Optimal testing for *NTRK* fusions clinically has yet to be established. In clinical trials for both larotrectinib and entrectinib, no single genomic test was utilized uniformly. Instead, a majority of patient were tested using either NGS or fluorescence *in situ* hybridization. Current testing algorithms suggest that a combination of fluorescence *in situ* hybridization, NGS, and immunohistochemistry are effective in identifying patients with *NTRK* fusions.[25–29] While no one diagnostic test was developed alongside either larotrectinib or entrectinib, development of an FDA-approved companion diagnostic is currently underway.

While the approvals of larotrectinib and entrectinib allowed their use in any cancer type meeting each drug's specific use criteria, it is important to note that these agents represent an approved therapy in rare cancers that have no alternative therapies available and a high prevalence of *NTRK* fusions. These cancers include mammary analogue secretory carcinoma, cellular or mixed congenital mesoblastic nephroma, and infantile fibrosarcoma.[29–31] This pathway to drug approval highlights the potential for drugs that are designed preclinically to have molecular, and not histologic targets. These two approvals also highlight the willingness of regulators to work alongside industry partners to develop processes in novel clinical situations to provide therapies to patients.

Commonalities in tissue-agnostic drug development

The development of pembrolizumab in patients with MSI-H/dMMR cancers as well as larotrectinib and entrectinib in *NTRK*

fusion–positive cancers represents novel drug/indication approval processes. Reviewing the common traits between these approvals provides insight into what the future of tissue-agnostic approvals may look like (**Table 3**). A striking similarity in the tissue-agnostic approvals of pembrolizumab, larotrectinib, and entrectinib include the FDA's acceptance of pooled data from multiple single-arm trials to grant accelerated approval. The FDA required substantial evidence of efficacy and safety for these agents based on direct or surrogate markers of clinical benefit. Here, all three drugs were approved based on their ORR and duration of response in clinical trials. In the case of larotrectinib and entrectinib, the rarity of *NTRK* fusions resulted in a lack of feasibility of a single, stand-alone analysis, thus necessitating a pooled analysis. Key to the acceptance of pooled trial data was consistent clinical response seen across individual studies. Clinical confirmation of strong preclinical and translational theory by consistent response without regard to patient age or tumor type bolstered the utilization of pooled clinical data. While the three currently approved tissue-agnostic drug development processes do not provide a single obvious pathway for the next generation of these approvals, lessons can be inferred from the shared characteristics between their approvals.

Following the accelerated approval of pembrolizumab, larotrectinib, and entrectinib as described above, each therapeutic agent was required to provide robust response outcomes in additional prespecified patient cohorts. These studies are yet to be completed; therefore, the next step in cementing the tissue-agnostic indications by the FDA will be eagerly awaited. While randomized clinical trials

Table 3 Comparison of tissue-agnostic therapy development processes

MSI-H/dMMR – Pembrolizumab	Shared	NTRK Fusions – larotrectinib, entrectinib
Prospectively identified biomarker	Strong scientific rationale	Drugs designed to target molecular biomarker
Genomic phenotype biomarker	Pooled analysis of clinical trials	
Clinical trials not designed for tissue-agnostic approval process	Consistent clinical response	Gene fusion biomarker
	Companion diagnostic test not developed simultaneously	Clinical trials designed for tissue-agnostic approval process
Retrospective and prospective analysis	Large single-arm postmarketing studies	Orphan drug status

MSI-H/dMMR, microsatellite instability-high/mismatch repair deficient.

may not be feasible for some studies enrolling multiple tumor types, large confirmatory postmarketing studies will remain important to confirming tissue-agnostic activity of targeted agents. The lack of a randomized comparator group makes a survival-based efficacy end point not possible, necessitating the use of surrogate end points like ORR. While the FDA has set a precedent that single-arm postmarketing studies are acceptable in instances where randomized clinical trials are not feasible, an alternative strategy could be the use of real-world data to confirm response of these therapies, such as in the TAPUR trial.[32,33] Studies such as this prospectively collect response on a case-based level to assess the efficacy of FDA-approved therapies in real-world practice. Additionally, these study types can help to extend the assessment of surrogate end points where survival-based end points are not possible, such as in the case of the aforementioned tissue-agnostic approvals. These three approvals would suggest that future approvals will require strong scientific rationale, consistent clinical response across studies intended to be pooled for analysis, and consistent clinical response in all tumor types studied, as well as follow-up to accelerated approval with a large confirmatory nonrandomized clinical trial.

CURRENT LANDSCAPE OF TISSUE-AGNOSTIC DRUG STUDIES
Novel clinical trial designs
Following the advent of molecularly guided therapy, novel clinical trial designs were developed to optimize patient selection while maximizing resources. Basket trials incorporate multiple trial arms which, in the case of tissue-agnostic drugs, nonrandomly assign patients to specific treatment based on their molecular profile. Basket trials were developed at the advent of tissue-agnostic therapies as a method of rapidly testing genetic mutation-drug matched therapy across cancer types in an expedient process. These original basket trials, including Molecular Analysis for Therapy Choice (NCI-MATCH; NCT02465060) and Targeted Agent and Profiling Utilization Registry (TAPUR; NCT02693535), are now yielding data regarding the efficacy of a number of targeted therapies across histologies with the results then guiding additional expansion arms and novel therapies and combinations. Additional large, National Cancer Institute (NCI)-sponsored basket and umbrella trials including the ALK Trial, Molecular Profiling-Based Assignment of Cancer Therapy (MPACT), and pediatric Molecular Analysis for Therapy Choice (pediatric-MATCH), are designed to test biomarker-directed therapy while allowing multiple biomarker arms to open and close within the trial as data become available.[34] Additional focused studies have been opened to

ask specific questions regarding the targetability of tissue-agnostic biomarkers. A selected list of active tissue-agnostic clinical trials is available in **Table 4**. The results of these trials will provide further insight into the future of tissue-agnostic therapies. While it is unclear if and which arms within current basket trials will be successful, this design aids in optimizing patient enrolment and gives opportunities to provide targeted therapy to patients while capturing the "n-of-1" extraordinary responders in a the setting of a clinical trial. Incorporating modern adaptive trial designs, including multistage, Bayesian, and aggregation studies can further incorporate improved signal finding while reducing the number of subjects necessary to power studies.[35]

Large scale multi-omic studies
To aid in the identification of novel, potentially targetable genomic alterations, large-scale efforts are underway to molecularly characterize as many cancers as possible. These data, after initial analysis, are often publicly available, and are utilized to aid researchers as a complement to smaller, focused research efforts. The Cancer Genome Atlas (TCGA), an effort of the NCI as well as the National Human Genome Research Institute (NHGRI) launched in 2006, is a landmark research network that has successfully molecularly characterized numerus samples. A recent collaboration between TCGA and the International Cancer Genome Consortium (ICGC) reported multi-omic data from 2,658 cancers including 38 unique cancer types.[36] Parallel to these efforts, genome scale CRISPR-Cas9 (clusters of regularly interspaced short palindromic repeats–CRISPR-associated protein 9) screens have provided a better understanding of potential biomarkers of drug sensitivity as well as opportunities for drug synergy in combination.[37] These large-scale efforts represent opportunities to identify the next generation of targeted therapies while, potentially, holding the key to finding the next tissue-agnostic drug target.

Emergence of mutational signatures
Cancer cells contain thousands of mutations in addition to identified driver mutations. Each mutational process leaves a characteristic imprint, or mutational signature, on the cancer genome. These mutational signatures can reveal prior exposure to toxins such as tobacco, ultraviolet light, radiation, and chemotherapy, as well as ongoing biological processes such as DNA repair pathways.[38] The ultimate mutational pattern is determined by the intensity, timing, and duration of toxin exposure and by the functional status of each DNA repair

Table 4 Active tissue-agnostic clinical trials

Target	Therapeutic Agent(s)	Selected Clinical Trials[a]
ALK	Entrectinib	NCT02568267
		NCT02650401
		NCT03375437
	Repotrectinib	NCT03093116
		NCT04094610
BRAF	PLX8394	NCT02428712
DNA Repair Deficieny	Atezolizumab/Rucaparib	NCT04276376
FGFR	Debio1347	NCT01948297
		NCT03834220
MSI-H/dMMR	HLX10	NCT03941574
	INCB099280	NCT04242199
	INCB099318	NCT04272034
	QL1604	NCT04326829
	Tislelizumab	NCT03736889
RET	Pralsetinib	NCT03037385
	Selpercatinib	NCT04280081
		NCT04320888
	TPX-0046	NCT04161391
ROS1	Entrectinib	NCT02568267
		NCT02650401
		NCT03375437
	Repotrectinib	NCT03093116
		NCT04094610
TRK	DS-6051b	NCT02279433
		NCT02675491
	Entrectinib	NCT02568267
		NCT02650401
		NCT03375437
	Larotrectinib	NCT02465060
		NCT02576431
		NCT02637687
		NCT03213704
		NCT03834961
	Repotrectinib	NCT03093116
		NCT04094610
	Selitrectinib	NCT03215511
		NCT04275960

ALK, anaplastic lymphoma kinase; BRAF, B rapidly accelerated fibrosarcoma; FGFR, fibroblast growth factor receptor; MSI-H/dMMR, microsatellite instability-high/mismatch repair deficient; RET, ret proto-oncogene; ROS1, c-ros oncogene 1; TRK, tropomyosin receptor kinase.
[a]Selected clinical trials identified on ClinicalTrials.gov on April 27, 2020.

pathway. The fractional contribution of each specific mutational process to the overall mutational signature varies and can be used to get a more global assessment of the tumor's biological history. Potential therapeutic uses of mutational signatures include impaired homologous recombination

DNA repair measured by HRDetect in predicting sensitivity to DNA damaging agents and APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) mutagenesis in predicting sensitivity to WEE1 kinase and ATR (ataxia telangiectasia and Rad3-related protein) inhibitors.[39,40] While single-gene biomarkers have been utilized to define sensitivity to therapeutic intervention, the more phenotypic measure of mutational signature holds promise for identifying potential pan-cancer drug sensitivities.

Identifying resistance mechanisms to targeted therapy

In tissue-agnostic drug development, molecular testing is vital for identifying and understanding primary and secondary drug resistance mechanisms. Historically, many studies have failed to capture lineage and tumor evolutionary processes leading to therapeutic resistance during preclinical drug development and clinical trials. The incorporation of longitudinal genomic sequencing of tumors throughout clinical trials has been utilized to identify lineage-specific, evolutionary or clonal mutational processes offering novel information that can be used to guide future trials. In searching for the next tissue-agnostic therapy, many instances of tissue-directed biology have been identified. For example, *BRCA1* and *BRCA2* mutations have been identified as unreliable tissue-agnostic biomarkers of PARP (poly (ADP-ribose) polymerase) inhibitor sensitivity.[3,41] In the case of targeting B rapidly accelerated fibrosarcom (BRAF) with BRAF/MEK (mitogen-activated protein kinase kinase) inhibitors, response agnostic of histology cannot be assumed.[42] Data from a phase I trial of the BRAF inhibitor vemurafenib in $BRAF_{V600E}$−positive metastatic CRC patients identified heterogeneous response to targeted therapy mediated by molecular feedback through epidermal growth factor receptor (EGFR), limiting BRAF inhibition in CRC but providing insight into the biology of $BRAF_{V600E}$−mutated colorectal cancer.[43] This ultimately resulted in the approval of the BRAF inhibitor encorafenib being approved in combination with the anti-EGFR monoclonal antibody cetuximab for $BRAF_{V600E}$−mutated colorectal cancer.[44] This example underscores the value of preclinical study exploring connections between biomarkers and clinical response. Secondary resistance mechanisms further complicate the applicability of biomarker-directed therapy. The discovery of acquired resistance in TRK inhibitors has led to the development of second-generation TRK inhibitors LOXO-195 and repotrectinib.[45] By incorporating longitudinal molecular testing, continued clinical-molecular study can provide insight into future opportunities for therapeutic development.

Genomic differences between cancer models and human disease can also drive misinformation in preclinical study. Better preclinical models that can accurately re-create the environment of the broad spectrum of clinical cancer types are necessary to better test the validity of targeted therapy, especially for tissue-agnostic purposes. Cancer demonstrates complex molecular feedback resulting in heterogeneous off-target therapy resistance. Better identification of similarities in cell lineage and histology will aid in identifying common oncogenic pathways for therapeutic targeting.

Multidimensional measures of tumor biology

Development of NGS has not been the sole area of evolution in defining the 'omic landscape of cancer. Advances in RNA-sequencing, methylomics, and proteomics have driven our understanding of the biology of cancer. Traditionally, targeted therapies have been designed based on a single molecular biomarker. With the expansive data available today, multibiomarker targeting is possible. The future of these technologies will incorporate methods to address the weaknesses of single timepoint testing. Tissue-agnostic trials represent the ideal scenario for getting a better understanding of the relevance of the total genomic / multi-omic picture of a tumor, as initial tissue-agnostic trials have shown that the molecular status of a single biomarker alone might not be enough to predict the response and resistance mechanisms in different tumor types. By utilizing longitudinal multi-omic assessment, a better understanding of an individual's cancer biology can be leveraged towards treatment.

MODERN CHALLENGES IN REVOLUTIONARY DRUG DEVELOPMENT

Undoubtedly, tissue-agnostic drug approvals in oncology represent a paradigm shift in the field. While it is likely that this shift will prove to be the catalyst for additional approval, further approvals will need to overcome a number of challenges. These challenges, in the opinion of the authors, are discussed here and in **Figure 2**.

Development of genomic technologies

Over the past few decades, incredible improvements in genomic technologies have rapidly shifted the bottleneck of these technologies, in terms of cost, time, and interpretation, from the benchtop to the laptop. Better multi-omic technologies require improved bioinformatics tools and an increase in bioinformatics-trained clinical scientists to identify molecular drivers of disease amenable to treatment. The rapid growth of the field, including microarray, NGS, nanostring, and single-cell technologies, has resulted in

an increasing amount of genomic data that has been generated in previous studies using now obsolete methods. These data are still highly valuable to inform future study; therefore, methods to incorporate old and new genomic data are needed.[46] Unraveling the clinical meaning of rare genomic variants, variants of unknown biological significance, and mutational signatures may provide insight on targetable variants and primary resistance mechanisms, and ultimately improve clinical care. In the rapidly moving field of genomics, equipping researchers and clinicians with better tools to deal with these challenges is vital to the future of precision medicine.

Cost and availability of genomic testing

The cost of genomic testing has fallen dramatically over the past decade. While incorporating genomic sequencing into clinical care has demonstrated clinical utility and even cost savings, willingness of payers to cover such testing is inconsistent.[47] This reduces the volume of patients receiving genomic testing for their cancers, thus reducing the pool of patients with genomic results available to identify opportunities for molecularly guided therapy. From a research perspective, the addition of a molecular/genomic entry criterion for a clinical trial greatly increases the cost and complexity of running said trial. Basket trials have been successful in increasing the potential available matched treatment arms to maximize the value of genomic sequencing for a patient but have also had to overcome the challenges of obtaining a recent tissue biopsy and enabling rapid enrollment and treatment for patients who often have aggressive cancers. For smaller studies, covering the cost of genomic testing remains a hurdle.

In the setting of advanced cancer, NGS-identified biomarkers are now incorporated into the standard of care of numerous cancer types in the first-line setting[48] and have also become a standard of care to optimize later-line therapy recommendations.[49,50] Clinically, it is important to weigh the amount of tissue needed for individual assays with the value of the clinical information provided by the
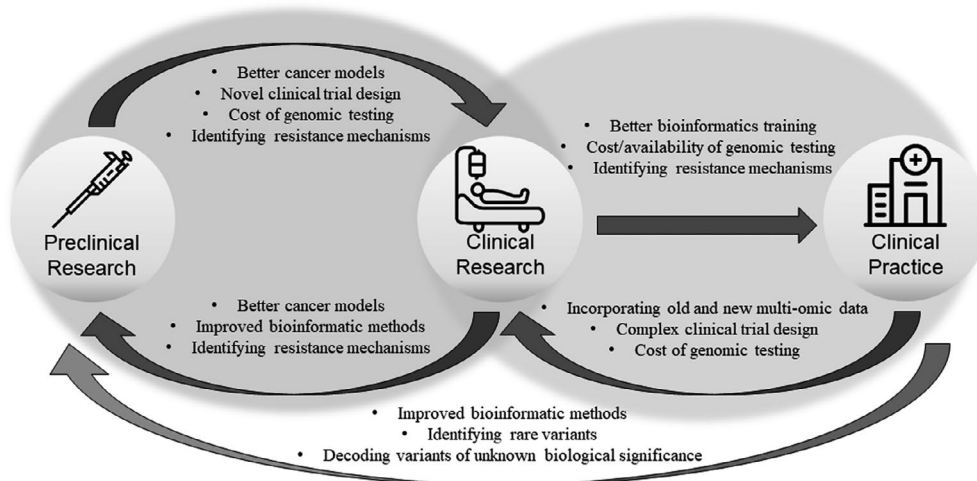

Figure 2 Challenges in the development of tissue-agnostic drugs. Tissue-agnostic drug development is complicated by a number of challenges in the preclinical and clinical research space, as well as in clinical practice. Challenges noted here include development of better cancer models, novel clinical trial design, improved bioinformatics training and methods, cost of genomic testing, identifying resistance mechanisms, incorporating old and new multi-omic data, identifying rare genomic variants, and decoding variants of unknown biological significance.

assay, as well as its cost and efficiency. Based on this, clinical-grade broad NGS panels with adequate coverage for treatment-related molecular drivers are becoming part of standard practice in modern cancer care. The ongoing development of clinical pathways derived from national and international treatment guidelines is underway at numerous institutions.[51] These clinical pathways incorporate recommendations for when to include more focused molecular testing, while balancing the limitations of laboratory test availability and efficiency in tissue procurement is required.[52]

CONCLUSION

Tissue-agnostic drug approvals represent a paradigm shift in drug development, which will continue to provide more precise therapy to patients. It is likely that tissue-agnostic drug approvals will continue to grow through enhanced understanding of cancer biology, improvements in technology, and translation of this knowledge into therapeutic targets. However, we must balance our enthusiasm for novel targets with evidence provided through clinical trials. The rationale for the biomarkers and therapeutic agents being investigated should be strongly supported by optimized preclinical models and thoughtfully designed to yield meaningful data. These data must be robust enough to be translated into clinical decision making as well as inform future clinical trials.

CONFLICTS OF INTEREST
C.M.W.: Consultant for Intermountain Healthcare and Jackson Genetic Laboratories Molecular Tumor Boards, employee of HCA Mission Hospital. All other authors declared no competing interests for this work.

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