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Chapter

Precision Medicine Concepts in T-Cell Lymphoma

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Abstract

Modern oncology witnesses an increasing number of new effective anticancer drugs targeting specific oncogenic pathways. Despite these advances, real-world experience with targeted single agents is disappointing since drug resistance usually occurs after a short time. This is particularly true for patients with refractory or relapsed T-cell lymphoma (TCL) who so far could not benefit from novel agents and demonstrate a short survival time of only 3 months. The novel genetic information gained from genome-wide high-throughput techniques has greatly improved our understanding of TCL. However, if precision medicine strategies are based solely on genetics, it runs into two major challenges: (1) the heterogeneity within the cancer of an individual patient and (2) the incomplete understanding of the degree of contribution of a specific mutation to a tumor phenotype. Next-generation functional drug screening (ngFDS) aims to address these problems. Studies that proof the clinical utility of ngFDS are currently limited. The following chapter aims to discuss recent advances of ngFDS and to line out its potential for TCL patients.

Keywords: T-cell lymphoma, precision medicine, genomics, transcriptomics, next-generation functional screening

1. Introduction

TCL is a heterogeneous group of rare lymphoid malignancies generally with dismal outcome [1, 2]. With current treatment options, a majority of patients do not achieve remission or experience relapse after completion of therapy [3–6]. Patients with newly diagnosed TCL are most commonly treated with anthracycline-based (CHOP-like) chemotherapy regimens, often followed by consolidation with high-dose chemotherapy and stem cell transplantation in eligible patients [1]. Patients with relapsed TCL have a dismal prognosis with a median overall survival of only 3 months [2]. Unfortunately, mechanisms of drug resistance in TCL leading to progression and relapse remain elusive, and predictive biomarkers do not exist, precluding clinical progress. Three major limitations have thus far hampered a systematic and causative understanding of drug resistance in TCL: (I) adherence to genetic studies and barriers in translating this genomic information into direct clinical benefit for patients, (II) as for functional analysis, a general adherence to cell lines that is prone to clonal artifacts and that not faithfully recapitulates relevant physiology, and (III) lack of considerable biobanks of viably frozen cells from fresh dissociated lymphomas. All three points are discussed in detail below.
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2. Precision medicine concepts

2.1 Advances and shortcomings of genetic studies

Predicting clinical treatment outcome from detailed characterization of patient material is one of the key challenges of modern oncology. The most so-called precision medicine approaches either rely on correlation of clinical outcome with molecular profiles such as genetic mutations [7] or attempt to reproduce the disease in an ex vivo model system and extrapolate from measured drug response to the patient outcome [8, 9]. Genetic approaches are state of the art for most diseases and have also been successful in some indications (e.g., targeting BCR-ABL with imatinib in chronic myeloid leukemia (CML)). TCLs, however, as most cancers, categorically differ from the rare monogenetic disease model and are driven by microevolutionary processes leading to broad genetic heterogeneity [10] and making a purely correlational logic extremely challenging [11].

Recent sequencing studies in TCL confirmed a set of genetic alterations in specific subtypes, including recurrent mutations in the epigenetic modifiers TET2, IDH2, and DNMT3A and the small GTPase RhoA in angioimmunoblastic T-cell lymphoma (AITL) [12–15], while JAK/STAT pathway alterations through mutations at various levels seem to be present across all TCL subtypes, particularly in anaplastic large cell lymphoma (ALCL) [16]. For the most common subtypes of TCL, including peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), AITL, and ALCL, genetic data are largely based on targeted DNA sequencing approaches focusing on mutations in pre-selected panels of genes, while unbiased sequencing approaches like whole exome sequencing are only available in small cohorts. Previous studies in ALK-ALCL utilizing immunohistochemistry and fluorescence in situ hybridization identified for ~38% of the patients with ALK- ALCL DUSP22- and TP63-rearrangements to be associated with good prognosis as for DUSP22- or with exceptional dismal prognosis as for TP63- rearrangements when receiving CHOP-like regimens [17].

Despite the significant amount of recurrent mutations in genes involved in DNA methylation like DNMT3A, TET2, or IDH2, no genome-wide epigenetic profiling has been reported in a sufficient amount of clinically well-annotated samples [12, 13, 18]. Therefore, we currently only can assume that broad epigenetic changes are frequent in TCL. In contrast to B-cell lymphoma, relatively strong responses to epigenetic modifying agents, such as HDAC inhibitors and methylation modifiers, are clinically evident [19–21].

Thus, there is evidence that in TCL the epigenome is of particular importance; however, systematic studies on epigenetic profiling in TCL are lacking and so are direct connections of genetic mutations in epigenetic modifiers and clinical responses to epigenetic drugs. A comprehensive characterization of genetic and epigenetic alterations and clinical response to specific drugs is highly warranted for TCL.

2.2 Advances and shortcomings of functional studies

Despite significant success to identify genomic alterations that might establish and drive hematologic malignancies, genetic studies face problems in translating genomic information into drug-able targets to directly benefit the patients. Results of personal medical efforts in cancer patients so far are sometimes promising but with the majority rather appearing disappointing [22–26]. In a landmark study that used each patient as their own control, Von Hoff et al. reported that 27% of patients with recurrent metastatic cancer of any kind had a 30% longer progression-free survival (PFS) with treatment selected on the basis of genetic profiling than they did with their previous treatment [25].
The first randomized trial of genomic-based precision medicine, the SHIVA study, did not use patients as their own control but investigated the effect of genetic marker-based targeted treatment comparing with treatment at physician’s choice in heavily pretreated patients with cancer. The SHIVA study failed to demonstrate a benefit for patients treated with genetic precision treatment [26]. We need to consider that our knowledge of how cancer genotype relates to its phenotype and of the complexity of the dynamic microevolutionary procedures that occur in an individual cancer is very limited [10].

Therefore, dynamic approaches that measure drug responses in cancer cells derived from patient biopsies promise to complement the static molecular measurements. Functional approaches could contribute important information to improve the selection of the right drug for the right patient at the right time. For instance, ex vivo chemosensitivity tests have been performed in samples of patients suffering chronic or acute leukemia [8, 27–30] and also in gut stem cell-derived organoids [31, 32], in breast cancer cell lines [33], and in xenografted mice [34, 35]. All these pioneering functional studies have not been integrated into clinical routine, but they provided proof of concept for ex vivo responses that may match clinical response. However, due to the extensive time of the assays used, the clinical benefits were limited [36–38]. First proof of concept data was obtained with functional screens detecting drug activity in cell cultures with luminescent assays that predicted activity and resistance to drugs in patients [9] and leading to initiation of a clinical trial for targeted therapy of relapsed acute myeloid leukemia [39].

In two recent reports, luminescent assay-based drug profiling has also been performed in a T-cell neoplasm and in T-cell prolymphocytic leukemia (T-PLL) [40, 41]. T-PLL is a rare, clinically heterogeneous neoplasm, which is treated with alemtuzumab-based induction in patients with symptoms, followed by consolidative stem cell transplantation [42]. There are no randomized controlled trials that inform the management of T-PLL; thus it is an area of clear unmet need. Boidol et al. used single-cell suspensions from fresh bone marrow, peripheral blood, or lymph node samples from 86 patients and exposed cells to 106 different compounds to perform functional drug screening after 72 hours [41]. Cancer cell-specific responses were calculated from individual dose-response curves, and tissue microarrays were generated for comparative protein expression profiling (Figure 1).

BCL-2 inhibitor venetoclax exhibited the strongest differential response for T-PLL (Figure 2). Ex vivo responses to venetoclax significantly correlated with BCL-2 protein expression scores but not with scores for BCL-2 gene family members BCL-XL and MCL-1. BCL-XL and MCL-1 expression scores demonstrated a significant correlation, while only MCL-1 appears to be inversely correlated with BCL-2 expression. T-PLL samples were among the entities with the highest BCL-2 scores, showing the most dramatic response to BCL-2 inhibition via venetoclax. Importantly, the second ex vivo drug screening report on T-PLL also found consistent activities of BCL-2 inhibition in T-PLL, thus confirming these results in an independent cohort [40] (Figure 2). It also demonstrates the reliability and reproducibility of functional assays.

It is noteworthy that luminescent assay-based functional assays can recapitulate differential responses to venetoclax of specific disease entities experienced in the clinic. High-resolution dose-response curves of venetoclax clearly distinguished CLL, AML, and T-PLL samples (Figure 3). As expected from clinical data, CLL samples demonstrated pronounced effects at already very discrete doses (50% inhibitory concentration [IC50], around 5 nM). In contrast AML samples responded at high concentrations (median IC50: 10 μM). Response curves of T-PLL samples were in the middle of AML and CLL samples (median IC50: 1 μM).
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Figure 1. Workflow: After sampling, single cell suspensions are used for high-throughput drug-screening. Formaldehyde conserved samples are processed for tissue microarrays for comparative protein expression profiling.

Figure 2. Cancer cell-specific responses were calculated from individual dose-response curves in two independent publications. In both, drug-profiling revealed that BCL-2 inhibitor venetoclax exhibited a significant differential response for T-PLL.
Trying to elucidate the mechanisms, we investigated the protein expression of other BCL-2 family members. Interestingly venetoclax treatment induced BCL-2 and BCL-XL protein expression in the two patients, since MCL-1 levels did not changed. Therefore BCL-XL upregulation could be a potential mechanism of
venetoclax resistance, and the additional use of drugs targeting BCL-XL or suppressing BCL-XL could be mechanistically synergistic (Figure 4).

Therefore, studies testing venetoclax with appropriate combination partners in T-PLL are warranted. Combination therapies are essential to overcome the resistance mechanisms that limit the long-term efficacy of conventional cytotoxic chemotherapies or targeted agents inhibiting single pathways. It is important to establish a workflow to systematically test multiple drug combinations and applied it to successfully identify synergistic combinations [43]. The main challenges to systematic large-scale drug-drug combination testing are that the number of two-drug combination remains in tens of thousands, thus limiting the numbers of combinations to be tested in primary patient material. To overcome this burden, the community continues to develop innovative computational approaches for preselecting sets of putative synergistic drugs involving network analysis, dynamic modeling, and high-content machine learning. However, based mainly on genetic data, only approx. 40% in silico predicted drug synergies are confirmed by ex vivo combination testing as demonstrated at the example of T-cell prolymphocytic leukemia (T-PLL) [44]. A reason for the rather low functional confirmation rate could be the low correlation between high-throughput ex vivo drug testing and mutation profiling [40]. It is therefore tempting to propose a smart way to preselect combination partners that are then analyzed in vitro.

2.3 Advances of single-cell functional studies

Luminescent assay-based functional assays have the big plus of high-throughput because full automation is more easily established. However, these assays cannot provide data at the single-cell level and thus no data on individual cell based functional information. Especially if the aim is to address the heterogeneity within one individual patient, i.e., to discriminate malignant and normal cells or to use complex co-culture systems, investigators could be interested in automated microscopic imaging technology. Minimally invasive protocols could provide drug-response information in co-culture systems. This could maintain leukemia and multiple myeloma cells for a longer cultivating time and enhance the screening capabilities of patient samples [45–47]. Snijder and colleagues investigated the clinical impact of a newly developed single-cell image analysis technology platform that operates using a combination of multi-parametric immunofluorescence and high-throughput automated microscopy [48]. In contrast to functional methods used previously, this next-generation functional drug screening (ngFDS) technique allows a fast tumor cell-specific quantification of biological parameters of millions of adherent and non-adherent individual cells with high sample efficiency, minimal sample manipulation, extensive automation, and fast turnaround times [49] (Figure 5).

The authors demonstrated that multi-parametric, image-based, immunophenotypic cytometry could reliably distinguish malignant cells from normal bystander cells in a high-content screening context. They showed how this approach can detect phenotypes across several cellular compartments, quantifying, for example, T-cell engagement by the bispecific, CD19-directed, T-cell engager blinatumomab in patient samples. They applied the method to patients with aggressive hematologic malignancies failing at least two lines of therapy and without further standard treatment options. These patients will usually receive either best available therapy or best supportive care or will be enrolled in clinical trials. Upon relapse, blood, bone marrow, pleural effusions, or excised lymph node biopsies were collected depending on the disease manifestation. The primary endpoints were to evaluate the feasibility of integrating ngFDS into the clinic and to assess clinical response
in patients who received a treatment according to ngFDS results as an individual healing attempt [49] (Figure 6).

This prospective single-center pilot study demonstrates that it is possible to integrate automated microscopy-based next-generation functional drug screening (ngFDS) for patients with aggressive hematologic malignancies into the clinical routine. Importantly, the ngFDS results suggested treatment regimens that lead to an improved ORR and longer PFS for patients than the last treatment regimen the patients had just experienced progression on (Figure 7). These results demonstrate that we are already in possession of a wide array of working chemotherapeutics and...
targeted inhibitors that in principle are capable of breaking drug resistance even in multi-refractory cancers, given that we identify the right drugs for each individual patient, at the right time during their treatment.

The study name is EXALT for extended analysis for leukemia/lymphoma treatment. It is a prospective non-randomized study with each patient functioning as their own control, thus allowing to determine the overall effect across heterogeneous disease entities and different therapies. The nonexistence of randomization could harbor a bias. In future studies testing ngFDS-guided therapies, randomization and physician choice are warranted.

Image-based quantification of drug effects with single-cell resolution in patient biopsies, as introduced here, represents a robust and clinically useful platform to assign powerful individualized therapeutic regimens. The strength of the approach resides in the statistical power derived from monitoring with computer-aided precision millions of individual functional events, i.e., single-cell drug responses, combined with the ability to discriminate cell types, allowing to score specific rather than general and averaged cytotoxic effects. The approach may be valuable for the personalized identification of clinically effective therapies for many other hematological malignancies, especially for rare diseases, like TCL. The selection of personalized therapy by ngFDS benefits from the ability to measure hundreds to thousands of drug exposures using small patient samples, where each ex vivo treatment includes healthy-cell-controls from the same patient sample. This allows for direct estimation of the therapeutic window, while the minimal ex vivo culturing of cells and compatibility with clinical diagnostic markers ensure fast and relevant feedback. Further, the platform can take advantage of the use of liquid biopsies where small target cells can be detected. These integrated drug response profiles, or “chemotypes,” of individual people are the culmination of the interplay between a number of molecular parameters of the responding cells, including not only the genetic, proteomic, and metabolic state of the cells but also the direct and indirect molecular interactions with other cells [48], recapitulating relevant physiological complexity. Such chemotypes may offer functional insight into the underlying health status of an individual, with potentially wide-ranging implications also in preventative and participatory medicine.

As shown here, ngFDS can provide clinically useful guidance in the absence of genetic information. However, the full potential of the approach will certainly be realized only through the synergy with genomic and other molecular patient profiling. This could lead to highly accurate personalized treatments, coupled with companion diagnostics, as well as powerful route to mechanistic elucidation of gene-to-phenotype relationships.
2.4 Outlook for precision medicine in TCL

Collectively, the combination of advanced functional strategies, chemical genetics, and phenotypic screening approaches to holistically chart and chemically probe mechanisms of drug failure opens fundamental new treatment opportunities for TCL patients. Phenotypic single-cell drug screening is a highly innovative approach that helps to identify sensitive drugs and proved to be effective in late-stage hematological malignancies [49]. The next step to achieve the real treatment goal of a “long-term remission” is to identify a systematic way to identify mechanistically grounded effective drug-drug combinations. Clinically fully annotated viable sample sets consisting of sufficient TCL samples will provide the basis to probe with the help of network analysis, dynamic modeling, and high-content machine learning a useful algorithm for individual combination treatment to tackle drug resistance up-front.

Novelty emerges from the innovative intersection of cutting edge technologies, such as an innovative drug screening pipeline at cellular resolution, the use of novel immune competent xenograft mouse models that recapitulate human immune response, and single-cell RNAseq applied in primary TCL cells upon multiple drug perturbations. Foremost, it will be important to probe these data sets with cutting edge bioinformatics and network medicine pipelines.

ngFDS will give hemato-oncologists a new tool to identify the most promising treatment combinations to help overcome resistance in otherwise refractory TCLs on a patient-to-patient basis. The success of ngFDS, added a new layer to how doctors can base treatment decisions, since it significantly improved the patients' outcome [49]. A rational guidance to pick the most appropriate combination partner promises to achieve long-term remissions.

I further expect that the established pipelines of ex vivo testing identified drug combinations will outline and instruct prospective clinical trials as has recently shown [41, 45] (approval and funding of a clinical study starting Q4 2018: venetoclax + ibrutinib in T-PLL (VIT-study;) Ph2 Study M18–803 (AbbVie) = the first global clinical trial for T-PLL).

Finally, ngFDS challenges the concepts of future clinical trial design. It requires a study design that provides the highest possible flexibility for experimental treatment to allow data-guided combination of targeted agents with antibody-based therapies for TCL. Larger prospective studies should focus on specific disease entities and randomize between arms of different treatment selection procedures to capture the full potential of functional assays for our TCL patients.

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