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High-throughput screening for drug discovery, targeting the cancer cell-microenvironment interactions in hematological cancers

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Abstract

Introduction: The interactions between leukemic blasts and cells within the bone marrow environment affect oncogenesis, cancer stem cell survival, as well as drug resistance in hematological cancers. The importance of this interaction is increasingly being recognized as a potentially important target for future drug discoveries and developments. Recent innovations in the high throughput drug screening related technologies, novel ex-vivo disease-models, and freely available machine-learning algorithms are advancing the drug discovery process by targeting earlier undruggable proteins, complex pathways, as well as physical interactions (e.g., leukemic cell-bone microenvironment interaction).

Area covered: In this review, the authors discuss the recent methodological advancements and existing challenges to target specialized hematopoietic niches within the bone marrow during

leukemia and suggest how such methods can be used to identify drugs targeting leukemic cell-bone microenvironment interactions.

Expert opinion: The recent development in cell-cell communication scoring technology and culture conditions can speed up the drug discovery by targeting the cell-microenvironment interaction. However, to accelerate this process, collecting clinical-relevant patient tissues, developing culture model systems, and implementing computational algorithms, especially trained to predict drugs and their combination targeting the cancer cell-bone microenvironment interaction are needed.

Keywords: Cancer cell -microenvironment interaction, drug combination, high content microscopy

Article highlights:

- The leukemic blast-bone marrow microenvironment interaction is an attractive target for future drug discovery, which holds potential of targeting drug-resistant cancer cell population in patients.
- The drug discovery process can benefit from the recent development in the culture models (e.g., in 2D and 3D co-culture models), screening technology, and artificial intelligence platforms as they can simultaneously mimic the interaction and allow high throughput screening.
- A wide variety of supervised machine learning algorithms have been developed to predict drug–combinations and drug responses that can be adopted to target the cancer-microenvironment interactions.

- There is critical need for implementing specific models for drug prediction which utilize the molecular information of the known genes involved in bone marrow-microenvironment interaction.

1. Introduction: Leukemias are heterogenous diseases characterized by a broad spectrum of molecular alterations that influence the patient's clinical outcomes. Further, they are driven by not only genetic or epigenetic alterations within different hematopoietic cell types but also due to the interaction of the hematopoietic cells with other non-hematopoietic cells (e.g., stromal, adipocytes, macrophages) in the bone marrow (BM) microenvironment [1, 2, 3]. For example, concomitant mutations and functional alterations in mesenchymal stromal cells (MSCs) of the bone marrow can cause oncogenesis in myeloid cells [4, 5]. Similarly, the malignant cells can also transform the MSCs within the normal niche to produce inflammatory cytokines and growth factors (e.g., IL-1β, IL-6) to support malignant cell expansion [6]. Further, these interactions can facilitate the transformed cells by immune evasion and protect them from chemotherapy (Figure1A) [3]. Hence, targeting the BM microenvironment in conjunction with leukemic cells can provide an effective therapy for leukemias such as Acute Myeloid Leukemia (AML) [7, 8].

The leukemic cell-microenvironment interaction involves diverse molecules, including cellular metabolites, receptors, junction proteins and other signaling molecules in the extracellular matrix (8, 9). Several such signaling molecules are druggable and can be targeted to interfere with leukemic-BM cells interactions (10, 11). Targeting these interaction pathways have identified multiple novel drugs undergoing clinical trials with some bring even approved in hospitals. For instance, in 2008, the FDA approved the first leukemic-BM cells interaction disruptor called Plerixafor (13). Plerixafor

blocks the 'CXCR4 chemokine receptor" disrupting the interaction between the bone marrow niche and leukemic blast cells leading to their mobilization from in the BM to peripheral blood (13). Plerixafor in combination with G-CSF is prescribed to mobilizes HSCs from the bone marrow to the peripheral blood for collection and subsequent autologous transplantation in patients with Non-Hodgking's lymphoma or multiple myeloma (12). Furthermore, both programmed cell-death protein 1 (PD-1) inhibitors (e.g., nivolumab, pembrolizumab) and anti-programmed death-ligand 1 (PD-L1) monoclonal antibodies (e.g., atezolizumab, durvalumab) are another promising treatment that target the cancer cell-T-cell interaction, which is being investigated in a variety of leukemias (14, 15). Drugs targeting inflammation, excessive reactive oxygen species (ROS), and angiogenesis are also under development phases (Table 1) for AML and other leukemias. The whole drug discovery field is witnessing a transformation due to the advent of multiple novel high-throughput technologies focused on characterizing the genomic makeup of patients, identifying different cell populations and score signaling interactions within the BM microenvironment (14). These techniques and resources can be adopted and modified to accelerate the drug discovery phase by targeting the leukemic cell-BM microenvironment interaction. Our focus is to describe experimental model systems, profiling techniques and use of publicly available computational tools for high throughput drug screening (HTS) and combination prediction targeting cell-microenvironment interactions in leukemia. We will suggest how these methods can be adopted for drug discovery targeting cell-microenvironment interaction.

2. Opportunities for drug discovery targeting cancer cell-microenvironment interaction:

We start by going through some of the recent development in the drug screening tools required for the drug discovery targeting the leukemic cell-microenvironment interaction. Rather than providing

a systematic review of all developed resources, we mainly focus on information sources required for HTS of big-chemical library in cancer including model systems, cell viability measurement and drug response prediction algorithms. For more comprehensive surveys of underlying biological mechanism, the reader is referred to recent reviews (16-20). We will discuss the use of these resources in Section 4.

***2.1 Ex-vivo* model system and culture methods for high-throughput drug screening (HTS) assay:** HTS is a widely used technique to assess the phenotypic effect of thousands of drugs on a pre-clinical model system (e.g., patient-derived primary cells, secondary cell lines) in short span of time with lower cost. Hence, HTS is used to explore the massive chemical spaces across both approved and investigational drugs, to identify effective and safer therapies to target cancer cells. Historically, cell-lines have been used as experimental models for HTS as they are easy to grow and handle in a 2-dimensional (2D) culture in the laboratory. However, these simplistic culture models don't consider the role of the other cell types present within the tumor microenvironment (e.g., macrophage), essential for the cancer cell's survival. Thus, the major bottleneck in the use of 2D cell-cultures is its inability to consider cell-cell interaction.

Alternative 2D and 3D co-culture-based models are now being developed, where cancer cells are grown together with other cells from its microenvironment, such as fibroblasts or stromal cells, that support cancer cell growth and development during ex-vivo drug screening (21-23). In 2D co-culture models, cells of different types are either mixed prior to plating and cultured together (21) or are separated by a physical barrier in the culture plate containing the growth media (24). These experiments are easy to handle, less time consuming and offers the possibility to study the effect of

drugs between different interacting cells compared to animal-based complex models. However, these co-culture-based models miss out on the blood vasculature and signaling interactions amongst other cell types present in the tissue. Hence the measurement may not represent the accurate drug response. Similarly, 3D-cell culture-based models (e.g., organoids, spheroids) are adopted for drug screening in both solid and hematological cancers as they can better model the cell-cell interaction in tumors rather than their 2D counterparts (25). For instance, spheroids are 3D-multicellular mass that can be developed from primary tumors or cancer cell lines when embedded within extra cellular matrix (ECM) hydrogels and resemble cancer tissue more closely due to their solid 3D structure. The 3D structure of spheroids offers a unique opportunity to model the growing cell's morphology, proliferation potential, and drug response in bone marrow and lymph node more closely (26-29). Further, spheroids are also considered more suitable model for studying hematological cancer, despite the circulating nature of the leukemia as spheroids are enriched for cancer stem cells (CSCs) which are responsible for drug resistance and relapse of the leukemic patients (30, 31).

Recent studies have shown that 3D co-culturing of AML cell lines with human bone marrow derived mesenchymal cells were a better model for drug resistance studies over cells cultured in 2D cultures or in suspensions (32, 33). Although these static co-culture-based 3D models provide a major improvement over the monolayer cell culture, it fails to model the vascularization and dynamic interaction between multiple immune cell types present in the bone marrow microenvironment. Therefore, missing the true effect of a drug response due to circulating chemicals, shear and mechanical stresses because of blood flow (33, 34). To address some of these short comings, 3D preclinical dynamic experimental systems such as Cancer-on-a-chip (COC) have recently been developed for hematological cancer. This consists of a microfluidic cell culture system with

multichannel that mimic the activities, mechanics, and physiological responses of entire organs, (or partly), representing an artificial organ like the bone marrow (35). For example, Zhao et al., developed a novel 3D-dynamic model consisting of primary human bone marrow stromal cells, osteoblasts and human leukemic cells cultured in a microfluidic collagen matrix platform where they assessed the effect of cytarabine on cell-cell interaction in an AML model (35). The 3D-dynamic model maintained similar viability of cancer cells at higher drug concentrations than 3D-static model, indicating a higher drug resistance in the 3D-dynamic model due to protection from microenvironment similar to the protective effect of bone marrow microenvironment in patients. However, the complexity of the COC decreases the total number of drugs that can be tested at a time in an experiment. Thus, limiting its use for HTS.

Furthermore, patient-derived-xenograft (PDX)-based animal models can be used to screen among a limited number of drugs for their effect on cell-cell signaling pathways in hematological and other solid cancers (36). PDX-based models can be useful for "mouse clinical trial" or MCT approach, where a panel of PDX are created using tumors from patient samples and are treated with a drug like phase II clinical trials (37, 38). The individual tumor response is analyzed to assess the efficacy and toxicity of drugs as well as to capture the inter-tumor heterogeneity of cancers. However, mouse PDX models gradually loose the human stromal cells originally present in tumors (dissected from patients) and are replaced by host stromal cells as the xenograft grows (39). This replacement by the murine stroma could confound the analysis of the human tumor-stroma interactions. The reason being that some mouse stromal cytokines might not affect human carcinoma cells in PDX model, failing to mimic the original tumor samples. This may limit the use of PDX models for tumor-

microenvironment interaction studies. The relation between physiological relevance and experimental throughput off different ex-vivo model has been shown in Figure 1B.

We suggest that different experimental model systems should be integrated and adopted at different levels of drug discovery targeting the cell-cell interaction. For example, 2D and 3D co-culture models are a suitable model for HTS as they are easy to handle (Table 2). Whereas leukemic cells derived from spheroid cultures may be relevant in targeted drug studies to investigate their effect on leukemic cell-bone marrow interactions as spheroids-like cell aggregates mimic the bone marrow microenvironment more closely.

2.2 Experimental techniques for HTS: Luminesce or fluorescence-based drug screening assays (e.g., ATP Assay of Cell Viability, Resazurin Reduction Cell Viability Assay) are common techniques used to measure biologically relevant parameters to predict the response of drugs on cancer cells (40-43). For example, the CellTiter-Glo® Luminescent Cell Viability Assay is a standardized method to determine the number of viable cells in a culture. The cell's viability is detected based on a luminesce signal from the luciferase reaction where the amount of ATP is measured from live cells using a luminometer. However, these assays produce the bulk readouts as averaged values for the effect of the drug over the viability of cell populations and ignore the underlying cellular heterogeneity of cancerous tissues. As a result, the signal can be derived from only an affected cancer-subpopulation which may not be the actual intended-target cell population within the sample. The method cannot discriminate for drug efficacy/potency over different interacting subpopulations in a microenvironment. Hence, this can lead to misinterpretation of the biological effect of drugs, especially in relapsed/refractory patients where drug-resistant cancer population drives the disease

progression with the help of microenvironment (44). The luminesce or fluorescence-based bulk assays may be inappropriate for drug screening focused on identifying new drugs targeted for tumor-microenvironment interactions, as it cannot quantify the effect of drugs on cell-cell interaction level (Figure 2).

As an alternative, image-based high-content screening (HCS) can be a potent strategy to discover drug targeting cancer cell-microenvironment interactions as shown in Figure 2. Imaging after simultaneous staining with multiple fluorescence colors can visualize complete cells belonging to different cell types, and their diverse cellular substructures, including physical cellular junctions (45-47). The generated image can be analyzed by sophisticated image softwares to quantify the individual morphological features (e.g., area, size, and shape of cells), and texture of cellular organelles. Further, fluorescence intensity from the colored proteins can be used to estimate cellular changes due to drug treatment among or within specific cell populations (48). Cell Painting is one such assay where six inexpensive dyes can be used to stain eight cell organelles and components present in a tissue sample (49, 50). These components are imaged in five channels, where each capture fluorescent light of a particular wavelength and can be used to assess the effect of drugs over different organelles (51). Similarly, mass cytometry imaging (MCI) offers a substantial multiplexing capacity for phenotypic profiling, where 40 proteins can be simultaneously stained. The images are acquired enabling visualization of a variety of distinct cell types in their native microenvironment within a tissue (52-54). Image-based drug profiling technology can be customized by performing multiple rounds of serial staining and destaining for markers relevant to a disease, which can be used to quantify the drug effect on various cell types (55, 45). However, the generated data from image-based screening can be highly complex and large. Hence, it can be challenging to analyze image data for big drug screening projects. Furthermore, the computational expertise required for image analysis from such

project is limited to certain academic groups and company. Hence, technological advances in image acquisition, processing, and analysis will be needed to establish HCS as a common and powerful tool for small molecule drug discovery (57).

High throughput flow cytometry is another powerful tool that is increasingly being used as phenotypic drug screening platform in both suspended and adherent cell systems after detaching from culture plate (58). High throughput flow cytometry can analyze one cell at a time from a heterogeneous cell population without needing to develop complex segmentation algorithms for data analysis, as required for imaging-based screening (59). It can quantify the different cell composition in patient samples and can easily be adopted to study the effect of drugs affecting cancer cell-microenvironment interactions. For instance, the recent development of HyperCyt® has enabled the use of flow cytometry as a powerful approach for HTS using multiplexed fluorescence intensity assays in both adherent and suspension cells. HyperCyt® can detect the effect of drug over various cell types in a high-throughput manner (60, 61). Furthermore, adoption of novel cell-cell interaction recording assays such as GFP-based Touching Nexus (G-baToN) (62) that label cells undergoing direct interactions using fluorescence proteins for high throughput screening can be helpful for drug screening at centers where fluorescence-based technologies are commonly used for drug discovery. The comparison of different experimental techniques that can be used for drug discovery targeting blast-microenvironment interaction has been summarized in Table 3.

2.3 Computational experimental model to score leukemic cell-microenvironment interaction: To develop drugs targeting cell-microenvironment interaction, we also need to quantify the proportion of various cell types present in the sample as along with the interaction between these cell-types at a gene or pathway level. Recently, single cell RNA-sequencing (scRNA-Seq) and mass cytometry time

of flight (CyTOF) are widely being used to identify different cell types in the bone microenvironment (63, 64). These techniques can quantitatively score the strength of interaction using gene or protein expression level involved in leukemic cell-microenvironment signaling (63). The interaction score for each pair of interacting proteins is usually calculated using the interacting ligand and their cognate receptor expression as input in a scoring function (63-65). In a recent study, Armingol et al. reviewed the method and tool used in cell–cell interactions assessment from transcriptomic data and the algorithms, such as those based on network model dissecting the HSC–niche interactions spatially and temporally (66). However, there is an urgent clinical need to develop a rational and systematic strategies for integrating these cell-cell interactions scoring technology (e.g., scRNA) with HTS for rapid identification of drug targeting leukemic cell-BM interaction in heterogenous drug-resistant patient samples.

Recently, we combined high throughput drug screening together with scRNA profiling to suggest safe and effective drug combinations targeting the functional diversity of heterogeneous tumors tissues (41). In another study, Kim et al. used scRNA along with drug screening in patient-derived xenograft models to optimize drug combination targeting metastatic renal cell carcinoma (67). Similarly, Anchang B et al. combined CyTOF with single-agent responses profiling using nested-effect modelling to suggest drug combinations that lead to maximal desired intracellular effects at the single-cell level in a heterogeneous tumor sample (68). However, more such computational-experimental approaches are needed that allow integration of drug screening with cell-cell interaction scoring technology to identify drugs targeting leukemic cell-BM integration.

2.4 Computational resources for drug response prediction targeting cancer cell-microenvironment interaction: More than 20 computational-experimental methods capable of suggesting safe and

effective anticancer drugs using the molecular information's from pre-clinical cancer models have been developed (40-43, 69, reviewed elsewhere,70). These models most commonly use single nucleotide variations, copy number variations, RNA expressions, methylation, and proteomics as input for drug-combination prediction. Despite reasonable prediction ability in the respective test datasets, many of the developed models finds limited use in the clinics as they tend to overfit the combination response in the training datasets. These models can provide valuable insights into drug combination mechanism of action and can also be used for marker discoveries (71,72). Some of these existing machine learning-based methods that use target-based approach to suggest combinations can be adopted for the discovery of novel and effective anticancer drugs targeting the cancer-microenvironment interaction (Table 4). For example, these models can be re-trained using smaller number of molecular features involved only in cancer cell-microenvironment interactions, which will reduce the feature size as compared to the patient samples. Hence, attenuating the overfitting problem. Furthermore, the use of prediction model that can capture nonlinear interaction between various cell types and signaling molecules in the microenvironment will be better able to predict novel drug targeting these interactions.

Apart from single drugs, drug combinations are being used as standard therapy for many of the cancers. Algorithms that can predict drug combinations targeting the cancer cell-microenvironment interaction will be highly valuable and useful. Cokol et al (73) developed a computational framework named Metabolism And GENomics-based Tailoring of Antibiotic regimens (MAGENTA) in the *E. coli* system that identifies synergistic or antagonistic drug combination targeting the *E. coli* and microenvironment interaction. It uses the chemogenomic profiles of individual drugs and metabolic perturbations in a cell, under different microenvironment to suggest the combinations. The method

can be adopted to identify synergistic or antagonistic drug combination targeting the cancer cell and microenvironment interaction (73). We also recently developed systematic computational-experimental approaches, scComb (41) and the Drug combination prediction and testing (DCPT) (74) platforms that identify drug combinations with optimum synergy-efficacy-toxicity balance to target heterogenous cancer cell populations. Although, some of the predictions from these computational-experimental methods may work via cancer-microenvironment interaction pathways, none of these algorithms specifically focus to identify drug targeting cancer-microenvironment interactions.

2.5 Challenges for drug discovery targeting cancer cell-microenvironment interaction: Although our understanding of the leukemic BM microenvironment in hematological malignancies has made substantial progress, we still have miles to go in understanding the leukemic BM. The development of large-scale drug screening program aimed at identification of drugs targeting the cancer niche is still in its infancy and there is a critical need for novel strategies, such as those capturing the oncogenic interaction, to eradicate malignant leukemic stem cells in hematological cancers. However, capturing, analyzing and targeting the underlying interactions in hematological malignancies pose a unique and substantial challenge, warranting careful, coordinated, and multidisciplinary investigation. We have identified the following areas with substantial challenges that need to be addressed in order to accelerate the existing efforts in drug discovery in the field.

2.5.1 Limited knowledge of underlying mechanism of blast microenvironment interaction: We now understand that BM microenvironment is a complicated ecosystem full of heterogeneity and can affect almost every aspect of cancer biology, further, they also influence the large number of healthy processes including hematogenesis and immunity (75-76). Treatment targeting tumour-

microenvironment interactions can cause severe side effects (e.g., arterial thromboembolic events, myelosuppression). Hence, the next generation of computational-experimental tools predicting drugs to target the leukemia-bone marrow interaction should prioritize regimens with optimum efficacy and toxicity. The prediction or design of such drug regimens will require a deep understanding of the correct physiological context how the interaction provide a benefit to tumor cells, as this can provide the foundation for tailoring a rational combination of existing drug to target the process. However, many of the intricate process underlying the leukemic-cell and bone marrow interaction has just beginning to be explored. Further, there is a lack of reliable and specific markers for different celltypes (e.g., MSC, endothelial) (77). The lack of such detailed knowledge poses a major challenge for the discovery of safe and effective drugs.

2.5.2 Technical hurdles: The current quantitative methods to score the extent of spatial and temporal interaction among niche cells require sophisticated techniques like imaging or single cell sequencing and complex scoring algorithm. Many of the interaction scoring algorithms are in its infancy and require information from ligand–receptor interactions databases, which is still incomplete and expanding. This scoring limitation may hinder the computational modelling of drug response and the validation of predicted regimens as determining whether drugs targeting the signalling modify their target in the niche could be difficult.

Continuous improvement of leukemic cell-microenvironment interaction scoring methods will likely advance computational prediction of safe and effective drug combinations as well.

2.5.3 Biobank facilities and collaboration: Both HTS program and experimental validation of predicted drug using require large amount of patient-derived-primary patient samples hematological samples. Further, depending on the research question, experimental validation may require isolated cells (e.g., stromal, T-lymphocytes) from BM aspirates, biopsies or lymph nodes. Storage and

preservation of these extremely valuable samples require established biobanks with special facilities and culture conditions require for these specific cell types. However, these kinds of facilities are not available to all the centers hence could prove a hurdle to drug discovery program targeting leukemic-microenvironment interaction. Biobanking facilities with appropriate collection, storage and culture condition need to be developed, refined, and standardized across different academic centers and industries. Apart from technical facilities, collection, processing, culture of such kind of sample requires careful planning, detailed communication, coordination, and extensive collaboration between clinicians and basic science researchers.

3. Conclusion: In summary, we described the experimental models, drug-screening techniques, and computational methods for drug discovery targeting the cancer-microenvironment interaction with leukemia as a model disease. The drug screening technology, culture method and computational algorithm has progressed considerably over the past few years leading to better hit-identification. Further, knowledge about dynamic and special interactions between leukemic cell-bone microenvironment interaction has improved substantially. These new technological development and accumulated knowledge provide a unique opportunity to target the interaction therapeutically which can lead to eradicate the leukemic stem cells. We suggest that different experimental model systems, should be integrated and adopted at different level of drug discovery targeting the leukemic cell-microenvironment interaction. For example, 2D and 3D co-culture models are the suitable model for high throughput screening of library involving large number of drug as they are easy to handle. On the other hand, patient's leukemic cells derived spheroid cultures may be relevant in targeted study of drugs identified through drug screening for their effect on leukemic cell-bone marrow interaction as spheroid like cell aggregates better mimic the BM microenvironment. Furthermore, integration of

recent cell-cell interaction profiling method along with HTS techniques can speed up the discovery of drugs targeting the leukemic cell-BM interactions. We also note the need for implementation of more computational models especially developed for prediction of drugs targeting cancer-microenvironment interaction in cancers is needed for accelerating the process.

4. Expert opinion

In this section, we highlight our opinion on drug discovery targeting the cancer cell-microenvironment interaction, specifically in hematological cancers as the large-scale cancer sequencing efforts have well characterized the genomic aberrations and related heterogeneity specific to each cancer type (78-82). Further, patient-derived primary tissues samples are easily available for drug screening in hematological cancers. These are invaluable to identify drug targeting specific interactions, either for initial drug discovery during the high throughput screening phase or for validation of drugs identified using other computational and experimental approaches (74, 82, 83). However, we argue that adoption of advancement in culture methods, screening technologies, and computational algorithms can further speed up the process of drug discovery targeting the cancer-microenvironment interactions. For example, the use of the co-culture-based model (e.g., 2D and 3D) side-by-side with patients-derived samples can ease the preclinical efficacy and toxicity testing of our constantly increasing pharmacological portfolio for rarely accessible tissues, such as lymph nodes and bone marrow. We believe along with others that testing both large-number of targeted and conventional therapies using drug testing assays in patient-derived *ex vivo* co-culture models, and later verified in patient-derived organoids (PDO) or xenograft (PDX) models *in vivo*, can enable the identification of high efficacy and low toxicity drugs, targeting cell-microenvironment interaction in a patient-selective way.

In addition to the experimental model system, there is also a need for flexible and fast assays that can capture the leukemic cell-bone marrow interaction quantitatively. Hence speeding up the early phase of identification of drug targeting the oncogenic cell-microenvironment signaling. Rather than using bulk assays to measure the drug efficacy in a screening, we argue that it is important to use assays that can carefully dissect the effect of drugs on various cell types and their interactions such as physical connections or communication signaling. The use of cell-cell interaction scoring techniques (e.g. scRNA, CyTOF, high-content-imaging) in drug-screening can identify drugs targeting cell-cell interaction and can also help to quantify the efficacy and toxicity, of multi-targeting mono- and combinatorial therapies on the different cell types in the pre-clinical model systems. Furthermore, their use can greatly reduce the extensive cost, time and risks associated with drug discovery process, before entering clinical trials.

We also suggest the need of implementing new artificial intelligence (AI) and machine learning (ML) models especially focused to predict drugs targeting cell-microenvironment interaction using the molecular features. Many *in-silico* drug prediction approaches have been developed, including AI and ML models, however, none of these methods have been specifically developed to predict drugs targeting cell-microenvironment interactions. Most computational studies use molecular information (e.g., mutation, RNA-Seq) to predict drug efficacy, yet many of their predictions fail at the validation stage and in clinics. These succumb because of overfitting data due to the curse of dimensionality and the numerous features along with small clinical samples (71, 84). We suggest that fitting models using only those features involved in cell-microenvironment signaling can reduce the curse of dimensionality problem, to some extent.

Although experimental models, cell-based drug testing technologies and cell-microenvironment interaction scoring techniques continue to improve, wider adoption of HTS for discovery of drug targeting cancer cell-microenvironment interaction can be held back by several logistic, regulatory, and financial issues. For instance, lack of solid tissues such as BM biopsies or lymph nodes in the established biobanks is a common hurdle for cell-microenvironment interaction as many of the biobank store blood tissues only. At the technological level, the biobanking of specific cell types such as stromal cells may require specific culture conditions that are different from the preservation of other hematological samples. Further, enrollment of patients, collection and storage of healthy and tumor samples requires careful planning, detailed communication, coordination and extensive collaboration between clinicians, surgeons, pathologists, and researchers. The sharing and reuse of pharmacogenomic data generated from these collected samples for new research or translational purposes needs clear regulatory legal guidelines as the process is often complicated by divergent legislations across countries. Furthermore, HTS, cell-cell interaction profiling technology and computational expertise required for drug screening is costly hence is out of reach for many academic laboratories which is slowing the drug discovery including drugs targeting tumor-microenvironment interaction. Taken together, while the drug discovery can be initiated through smart adoption of existing technology, the process can be speeded up by solving several additional biological and logistics hurdles.

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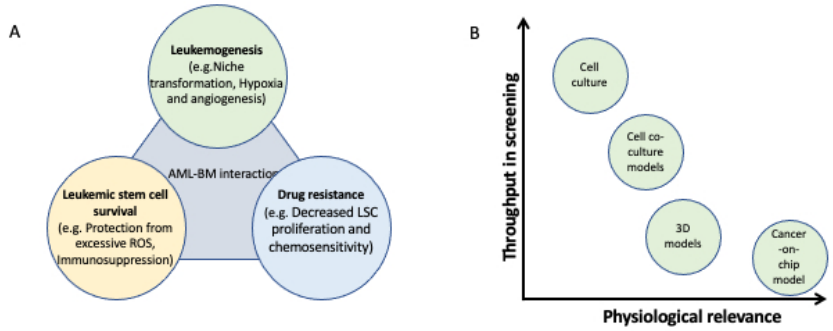

Figure 1: (A). Thematic diagram showing the effect of leukemic cell-bone marrow interaction on leukemogenic, leukemic stem cell (LSC) survival and drug resistance. (B) Diagram showing the relation between physiological relevance and throughput of experimental models used for during drug screening.

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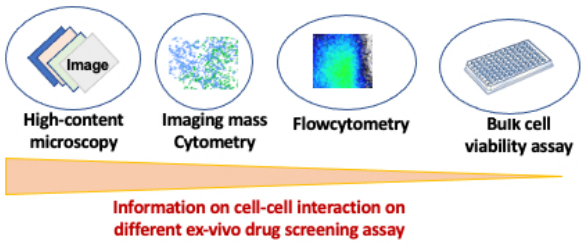

Comparison of the different ex-vivo drug screening assays on the basis of cell-cell interaction information

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Table 1: List of ongoing clinical trials for targeting leukemic cells-microenvironment interaction in AML

Interventions	Mechanism	Conditions	Clinical Trial Reference (Phase)
Crenolanib| Cytarabine| Mitoxantrone| Placebo Oral Tablet| Fludarabine| Idarubicin| G-CSF	Inflammatory pathway	Relapsed/Refractory Acute Myeloid Leukemia With FLT3 Activating Mutations	NCT03250338 (3)
Decitabine, Homoharringtonine, Aclarubicin, Cytarabine and G-CSF	Stromal cell–mediated protection of blast apoptosis	Acute Myeloid Leukemia|Induction Chemotherapy	NCT04083911(3)
Cytarabine| Daunorubicin| Uproleselan	Angiogenesis	Acute Myeloid Leukemia	NCT03701308 (2/3)
Uproleselan| Placebo	Angiogenesis	Acute Myeloid Leukemia	NCT03616470 (3)
Magrolimab| Venetoclax| Azacitidine| Cytarabine| Daunorubicin| Idarubicin| Steroidal Eye Drops	Recognition of blast by immune cells	Acute Myeloid Leukemia	NCT04778397 (3)
Homoharringtonine| Azacitidine	Stromal cell–mediated protection of blast apoptosis	Acute Myeloid Leukemia	NCT04248595 (3)
Galinpepimut-S| Best Available Therapy	Recognition of blast by immune cells	Acute Myeloid Leukemia	NCT04229979 (3)

CAR-T CD19	Recognition of blast by immune cells	Acute Myeloid Leukemia	NCT04257175 (2/3)
CD123/CLL1 CAR-T Cells	Recognition of blast by immune cells	Relapsed/Refractory AML	NCT03631576 (2/3)

Table 2: Comparison of *ex-vivo* model system and culture methods for high-throughput drug screening (HTS) assay

	Normol 2D culture	2D co-culture	Spheroids	Organoids	Cancer on chip	PDX	Animal models	Patient tumors
Physiological relevance	Low	Low	Medium	Medium	Medium	High	High	High
Throughput	High	High	Medium	Medium	Low	Low	Low	Low
Availability to labs	High	High	Medium	Medium	Low	Low	Low	Low
Cost	Low	Low	Medium	Medium	High	High	High	Low

PDX: Patient-derived xenograft model, 2D: 2-dimensional, 3D: 3-dimensional

Table 3: Comparison of experimental techniques commonly used for HTS for their possible use in drug discovery targeting cancer cell-microenvironment interaction

	Luminesce or fluorescence-based drug screening assays in cell culture	Touching Nexus	Mass cytometry imaging	Image-based high-content screening
Throughput	High	Low	Medium	Low
Physiological relevance	Low	High	Medium	High
Diffuculty in data analysis	Easy	Easy	Medium	Difficult
Availability	Common	Rare	Rare	Medium

Table 4: Drug-combination predicting algorithms that can be adopted to suggest combinations targeting cancer cell-microenvironment interaction

Methods	Data input	Combination prediction approach
scComb (41)	ScRNA profile, ex-vivo single drug response, drug-target information	Predict drug combination response using target expression level of involved drugs using an XGBoost model trained on single drug response and its target.
Metabolism And GENomics-based Tailoring of Antibiotic regimens (MAGENTA) (73)	Single drug response under different gene knockout conditions	Predict drug combination response using single agents' response under different genetic knockout conditions, and a random-forest model.

Drug combination prediction and testing (DCPT) platform (74)	Exome-sequencing, bulk-RNA-sequencing, ex-vivo single drug response in cancer patients and healthy controls	Predict drug combination response using target expression level of involved drugs and mutation profile as input using a random-forest model trained on single drugs' response and their target.
Probability ensemble approach (85)	Uses 6 target and structure-based information to calculate drug similarity (e.g. protein-protein interaction) and combine them using a Bayesian network into a likelihood ratio (LR) that represents its probabilistic similarity to the known interaction.	Combinations are prioritized based on their similarity with existing combinations.

DrugComboRanker (86)	Disease genomic profiles and gene expression profiles before and after drug treatment	Prioritized synergistic drug combinations using drug functional network and a Bayesian non-negative matrix factorization approach.
TranSynergy (87)	Drug-target information, gene expression or gene dependency	Uses transformer boosted deep learning model to predict combinations.
SynerDrug(88)	Drug target interaction, protein-protein interaction, and drug chemical fingerprint as input	Uses gradient tree boosting to predict drug combinations using probability distribution vectors of occurrence of drug combination target in a heterogenous network constructed from multiple sources (e.g., protein-protein, protein-drug interaction).
Ranking-system of Anti-Cancer Synergy (89).	Gene expression profile	Uses drug targeting networks and transcriptomic profiles to suggest drug combinations in cancer.

Combinatorial Drug Assembler (CDA) (90)	Gene expression profile	Drug combination suggestion by matching differentially expressed genes (e.g., between healthy and patient samples) with differentially expressed genes on drug treatment.
DrugComboExplorer(91)	DNA-seq, gene copy number, DNA methylation and RNA-seq data, drug pharmacogenetic data	Dysregulated driver signaling networks are identified using non-parametric, bootstrapping-based simulated annealing and later Bayesian factor regression approach is used on the network to identify drugs whose targets are enriched in the network.
Pang et al (92)	Drug target network, gene expression	Suggest drug combinations with complementary mathematical algorithms: Balanced Target Set Cover (BTSC) and Minimum Off-Target Set Cover (MOTSC).