Editorial

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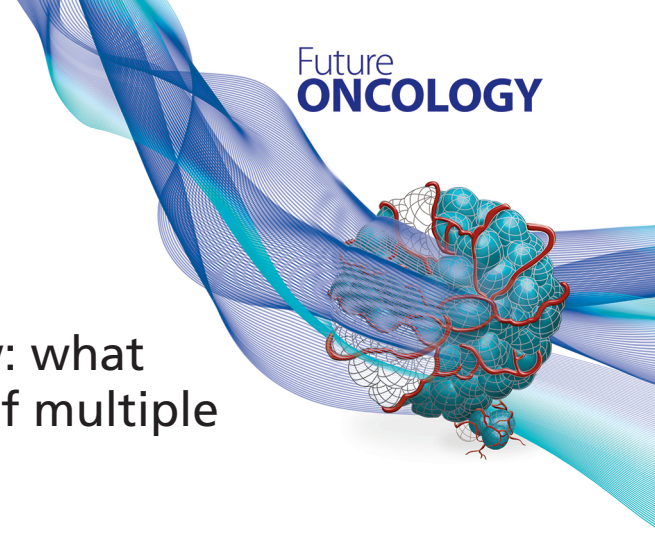

3D tissue-engineered bone marrow: what does this mean for the treatment of multiple myeloma?

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"The 3DTEBM will be evaluated in a clinical trial at Washington University School of Medicine in Saint Louis including MM patients, which will point out if this novel technology will be effective in predicting therapeutic efficacy in MM"

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Multiple myeloma (MM) is a hematological malignancy characterized by the proliferation of plasma cells in the bone marrow (BM), which remains incurable because most patients relapse or become refractory to the treatments [1]. The American Cancer Society's estimates for MM in the USA for 2016 about 30,000 new cases will be diagnosed and about 12,500 deaths will occur. Newly diagnosed MM patients with standard-risk disease are associated with a median overall survival of about 4 years, whereas high-risk disease MM patients are associated with a median overall survival of about 2 years [2]. Despite the introduction of promising novel agents, such as immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs), 40% of newly diagnosed patients do not respond to therapy and more than 90% of relapsed/refractory patients develop drug resistance [3,4]. The unsatisfactory results in the clinical outcome point to fundamental problems in our biological models for drug development.

Classic tissue culture screenings to assess drug efficacy rely on cultures of MM cell lines neglecting the interaction of MM cells with the BM microenvironment, which was shown to play crucial roles in disease progression, metastasis, and drug resistance in MM [5–8]. Some literature describe cocultures of MM cells with stroma mostly derived from normal subjects (such as HS-5 cell line); however, the effects of normal BM stroma on MM progression was different from, and sometimes opposite to, MM-derived stroma environment [9]. This implies that most of these MM–normal stroma cultures fail to recreate the malignant environment. Therefore, there is a need to develop models with MM-derived microenvironment. The BM niche is a 3D structure with functionally differentiated niches where the oxygen and drug concentrations gradually changed [10,11], while classic 2D tissue culture systems do not recapitulate such gradients. Another important limitation of classic 2D tissue culture models is that they rely on limited number of cell lines ignoring the highly variable genetic and epigenetic background of the MM

Future Medicine

patient population [12]. The discrepancies between drug efficacy in laboratory settings and the unsatisfactory clinical outcomes can be explained based on all the previous restrictions of classic tissue culture screenings.

Significant advances have been made in recreating the myeloma microenvironment in the last decade. Synthetic 3D models have allowed recreating relevant 3D features of the BM and its interactions [13–15], and the implantation of fetal bone chips in SCID mice have resemble even better the BM physiology [16]. In addition, *in vivo* xenograft models of human MM cell lines in SCID mice resembled the interaction of human MM cells with mouse microenvironment [17], while the C57BL/KaLwRij mice represent a syngeneic murine model of MM [18].

We have developed a novel patient-derived 3D tissue-engineered BM (3DTEBM) culture model derived from BM supernatant of MM patients and autologous cells from the same patient, which recapitulated the BM microenvironment [19]. Unlike the previous 3D models developed from exogenous materials, the 3DTEBM used the fibrinogen (a natural component of blood plasma and BM supernatant) to develop the 3D scaffolds that support the growth of BM components (MM and other accessory cells). The 3DTEBM is derived from the BM supernatant of MM patients, which includes all the growth factors, enzymes and cytokines naturally found in the MM microenvironment of a particular patient which will better represent the BM niche in MM [19,20].

The 3DTEBM was the first system to allow proliferation of primary MM cells *ex vivo* for several weeks and induced drug resistance in MM cells. The 3DTEBM recapitulated the polarized structure of the BM niche showing a more hypoxic niche with more stroma in the bottom of the scaffold and more proliferative cells (resembling the endosteal niche far from the blood vessel), while a more normoxic niche with less stroma, more endothelial cells and less proliferative cells closer to the surface (resembling the endosteal niche closer to the blood vessel) [10]. The 3DTEBM was able to successfully overcome several limitations of previous tissue culture screenings by recreating human MM cells interactions with their malignant microenvironment, simulating the drug gradients and hypoxic profiles of the BM niche and taking into consideration the individual heterogeneity between MM patients [19]. The 3DTEBM is a more physiologically relevant 3D culture system to accurately recapitulate the complex biology of BM microenvironment in MM, and is meant to be the next-generation tool to assess the role of the tumor microenvironment and its implication in progression and drug resistance in MM.

In addition to the biochemical and physical structure aspects of the BM niche, personal heterogeneity between patients is a critical factor. MM patients can be divided into numerous subgroups based on their genetic profile, and treatment responses can vary wildly between these groups [12]. With the lack of models to support progression of primary MM cells, and the extensive use of cell line-based tissue culture models, it is impossible to demonstrate the complexity of the individual heterogeneity of the MM patients. Since the 3DTEBM cultures were able to support proliferation of primary MM cells (from fresh and viably frozen samples), it provides a better tool to understand the pathophysiology of the disease, as well as the mechanism of drug resistance. Furthermore, it will allow drug screens on patient samples before the start of the clinical treatment to assess the sensitivity of MM cells to a wide array of drugs and provide potential personalized therapeutic options for the individual MM patients that the 3DTEBM was developed from, based on the biological characteristics of the patients own disease. The work flow will be able to isolate the BM supernatant from the patient, tissue engineer the BM in the laboratory, treat with a spectrum of anticancer drugs to determine which drugs are most effective, to finally provide the results to clinicians and their patients. The studies will be performed at any time in the course of treatment, allowing the assessment of new therapeutic options when a patient becomes resistant to a particular drug. Therefore, 3DTEBM cultures will provide a tool for prediction of the therapeutic response of MM patients *ex vivo*, which will help the clinician to design a better therapeutic plan for every individual patient based on the drug responses seen *in vitro* on a rapid timescale to guide therapeutic decision-making.

Moreover, the 3DTEBM model provides a unique option for drug development, to test the efficacy of novel drugs on primary patient samples, and investigate the effect of these drugs on the MM cells as well as their BM microenvironment. Particularly, this system will have a high potential especially for testing drugs that need the presence of other cellular components such as drugs affecting the immune system, checkpoint inhibitors, monoclonal antibodies and cellular therapy (CAR-T cells).

This model provides a paradigm shifting concept for biological research, drug development and personalized medicine in MM. Since current methods to assess potential cancer treatments are cumbersome, expensive and often inaccurate; this model offers a method to rapidly test interventions for cancer treatments and the personalized drug screenings will select screening strategies to lower the risk of MM patients, match patients with treatments that are more likely to be effective and ultimately increase efficacy and reduce mortality.

The 3DTEBM will be evaluated in a clinical trial at Washington University School of Medicine in Saint Louis including MM patients, which will point out if this novel technology will be effective in predicting therapeutic efficacy in MM. Moreover, we are working on further extending the same concept to other diseases, since the BM microenvironment was shown to play a crucial role in other hematologic malignancies such as leukemia and lymphoma, as well as in solid tumor bone metastasis.

Financial & competing interests disclosure

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