In vitro Assessment of Potential Hematotoxicity of ADC Drugs

Xiaomin Du[1], Qi Hu[1], Suhong Zhang[1], Pengfei Gao[1],Fang He[1]*

1Pharmaron Beijing Co., Ltd. No.6, Taihe Road, BDA Beijing, China

bd@pharmaron.com | www.pharmaron.com | booth #2134

Introduction

- Antibody-drug conjugates (ADCs) are transformative cancer therapeutics that deliver cytotoxic payloads with antibody-guided precision. However, their clinical utility is constrained by off-target hematotoxicities—such as neutropenia and thrombocytopenia—which frequently emerge as dose-limiting adverse events linked to unintended hematopoietic cell targeting or non-specific payload release.
- This study assesses the hematotoxic potential of major ADC classes across human CD34+ hematopoietic stem/progenitor cells (HSPCs) and differentiated erythroid, myeloid, and megakaryocytic lineages. Using cell viability and colony forming assay as key readouts, we aim to uncover lineage-selective vulnerabilities. These insights will support the rational design of safer ADCs and the optimization of clinical dosing strategies to mitigate hematological toxicities.

Methods

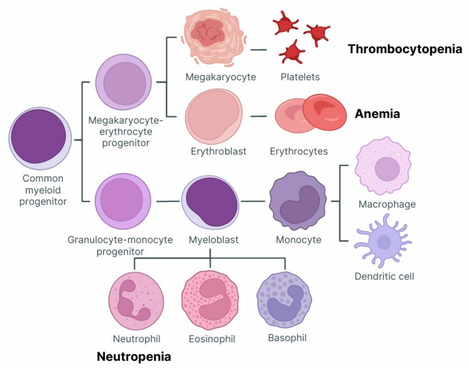
- Human CD34⁺ cells were cultured in StemSpan SFEM II (STEMCELL) with cytokines to preserve stemness, and then differentiated into erythroid, myeloid, and megakaryocytic lineages using specified cytokine cocktails for the required durations.
- Four clinically approved ADCs representing distinct payload classes were evaluated: trastuzumab vedotin (MMAE class), trastuzumab deruxtecan (DXd class), trastuzumab duocarmazine (DM class), and trastuzumab emtansine (DM1 class). CD34⁺ hematopoietic cells were treated with the indicated ADCs during lineage differentiation for 7–10 days. Cytotoxicity was assessed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega).
- For clonogenic assays, treated CD34⁺ cells were plated in methylcellulose-based medium optimized for hematopoietic colony formation. After 14 days of incubation, BFU-E (erythroid burst-forming units) and CFU-GM (granulocyte-macrophage colony-forming units) were scored manually based on morphological criteria under an inverted microscope.

Conclusion

- This study demonstrates that primary CD34+ HSPCs and their differentiated lineages provide a robust in vitro model to assess hematotoxicity potential for ADCs.
- This model enables early identification of lineage-specific risks and provides a basis for the preclinical development of ADCs, guiding payload selection and target validation to minimize hematotoxicity. Future work will expand to include more ADCs and investigate payload release kinetics in lineage-specific niches.

1

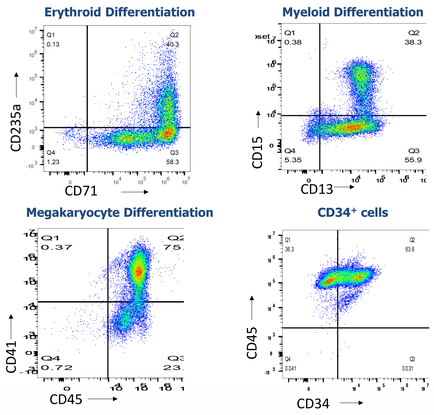
Hematotoxicity is a Common Adverse Event in Antibody-Drug Conjugate Therapy



Hierarchical development of hematopoietic cells during normal differentiation of bone marrow–derived hematopoietic stem/progenitor cells toward lineage-committed mature cells, together with their associated hematological toxicities.

2

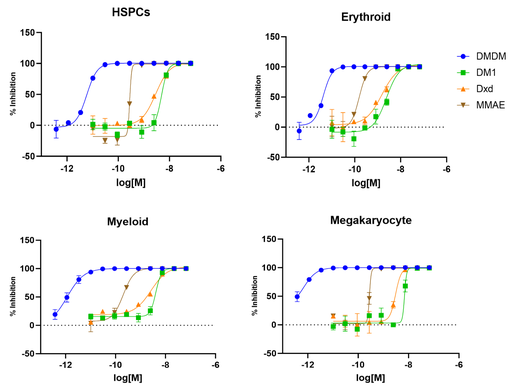
Establishment of *In vitro* Hematopoietic Differentiation System



Human CD34⁺ HSPCs were differentiated toward the erythroid, myeloid, and megakaryocytic lineages and subjected to immunophenotypic characterization via flow cytometry. CD71 and CD235a for the erythroid lineage, CD13 and CD15 for the myeloid lineage, and CD45 and CD41 for the megakaryocytic lineage.

3

Payloads Exhibit Lineage-Specific Cytotoxicity

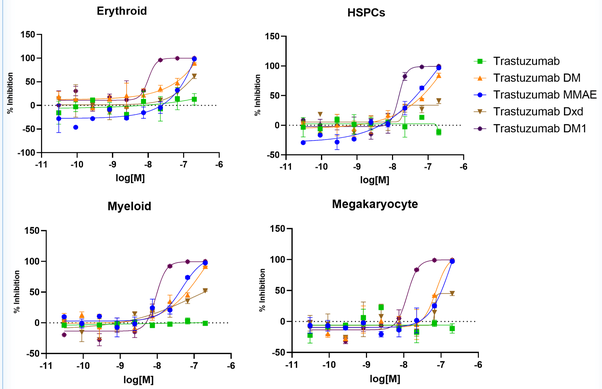


	Cytotoxicity EC_{50} (nM)			
	HSPC proliferation	Erythroid differentiation	Myeloid differentiation	Megakaryocyte differentiation
DMDM	0.006	0.004	0.0012	0.00068
DM1	4.84	2.37	4.08	6.92
DXd	2.88	1.50	2.62	3.03
MMAE	0.28	0.16	0.20	0.28

DMDM demonstrated preferential cytotoxicity toward the megakaryocytic lineage, while DXd and DM1 displayed higher toxicity toward the erythroid lineage. MMAE exhibited no lineage-restricted cytotoxicity.
DMDM: Duocarmycin DM; DM1: Mertansine; DXd: Exatecan derivative; MMAE: Monomethyl auristatin E.

4

Trastuzumab ADC payloads Differentially Impact Lineages in HSPC Cultures

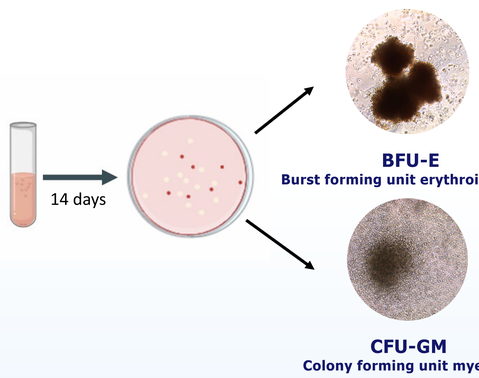


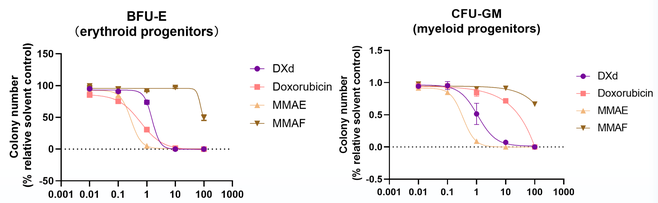
	Cytotoxicity EC_{50} (nM)			
	HSPC	Erythroid	Myeloid	Megakaryocyte
Trastuzumab	>200	>200	>200	>200
Trastuzumab-DM	52.83	70.01	52.76	73.37
Trastuzumab-DXd	>200	191.70	94.09	>200
Trastuzumab-DM1	14.45	10.11	9.31	12.45
Trastuzumab-MMAE	25.76	68.32	46.93	82.77

Trastuzumab-DM1 exhibited dominant cytotoxicity across HSPCs and three lineages, trastuzumab-DXd showed reduced toxicity toward all lineages.

5

The Colony Formation Assays can Effectively Detect Toxicities of Payloads





	Cytotoxicity EC_{50} (nM)	
	BFU-E	CFU-GM
DXd	1.54	1.13
Doxorubicin	0.60	15.89
MMAE	0.28	0.34
MMAF	74.90	24.89

MMAE exhibited higher cytotoxicity compared with DXd, Doxorubicin and MMAF. Doxorubicin showed preferential cytotoxicity toward the erythroid lineage relative to the myeloid lineage. MMAF: Monomethylauristatin F.