

Introduction

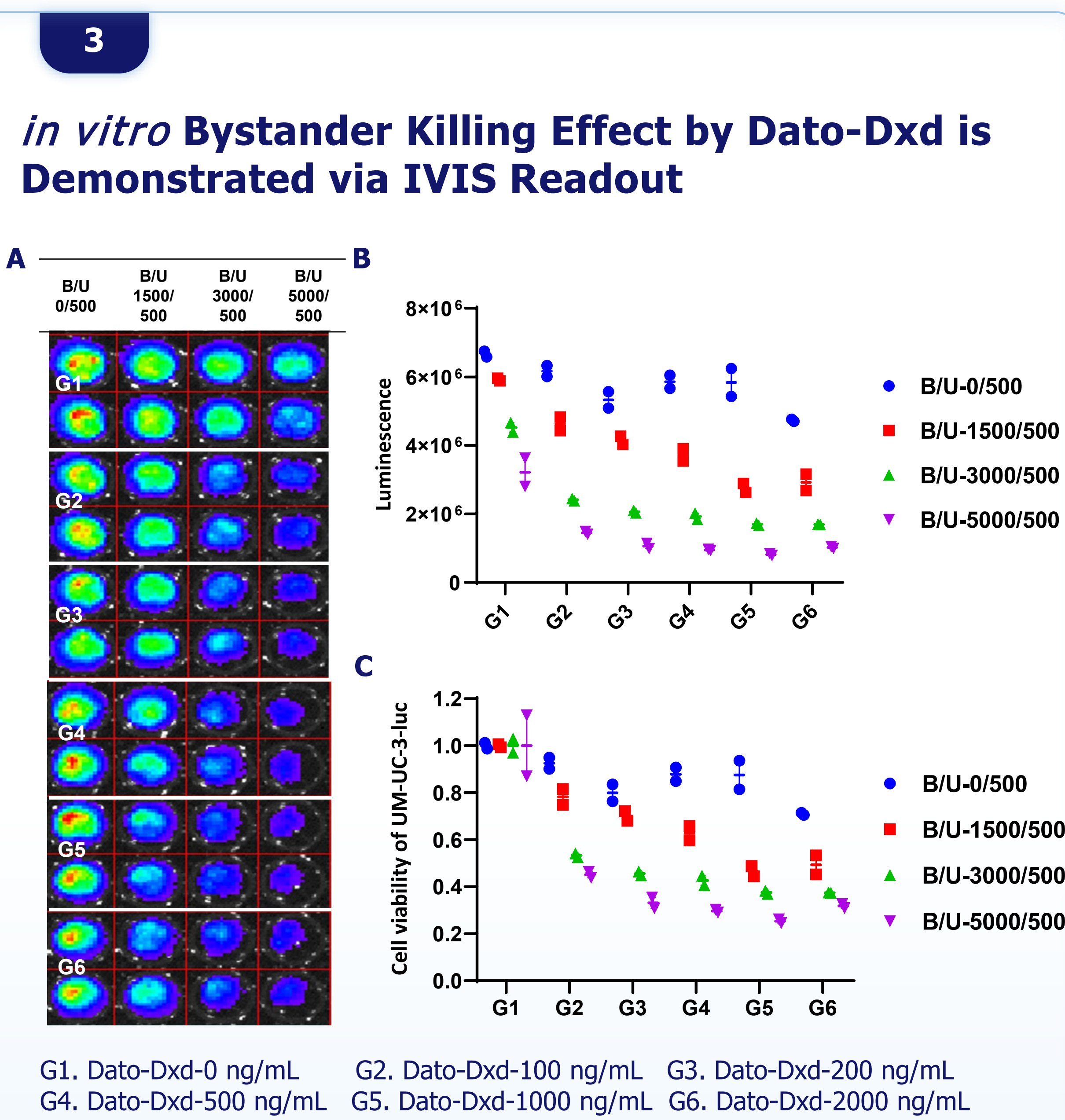
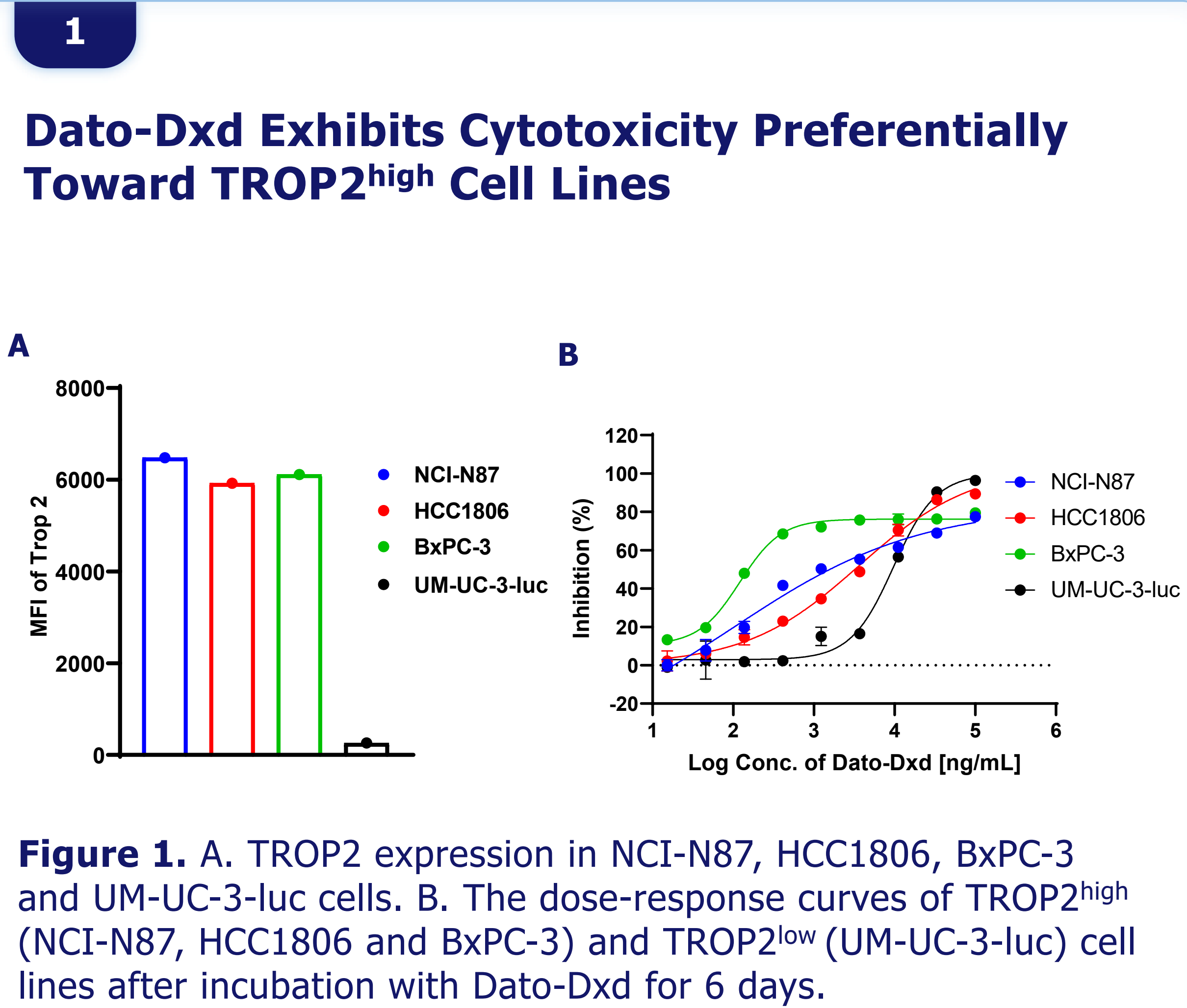
- Antibody–drug conjugates (ADCs) represent a promising class of drugs owing to their efficient and specific drug delivery.
- Some ADCs induce a bystander killing effect, mediated by soluble toxic payloads, gap junction intercellular communication, immune-mediated mechanisms, or Fc-mediated effector functions. This effect can eliminate not only antigen-expressing tumor cells but also adjacent antigen-negative cells.
- There is a critical need to develop systems that comprehensively evaluate the bystander effects of ADCs.

Methods

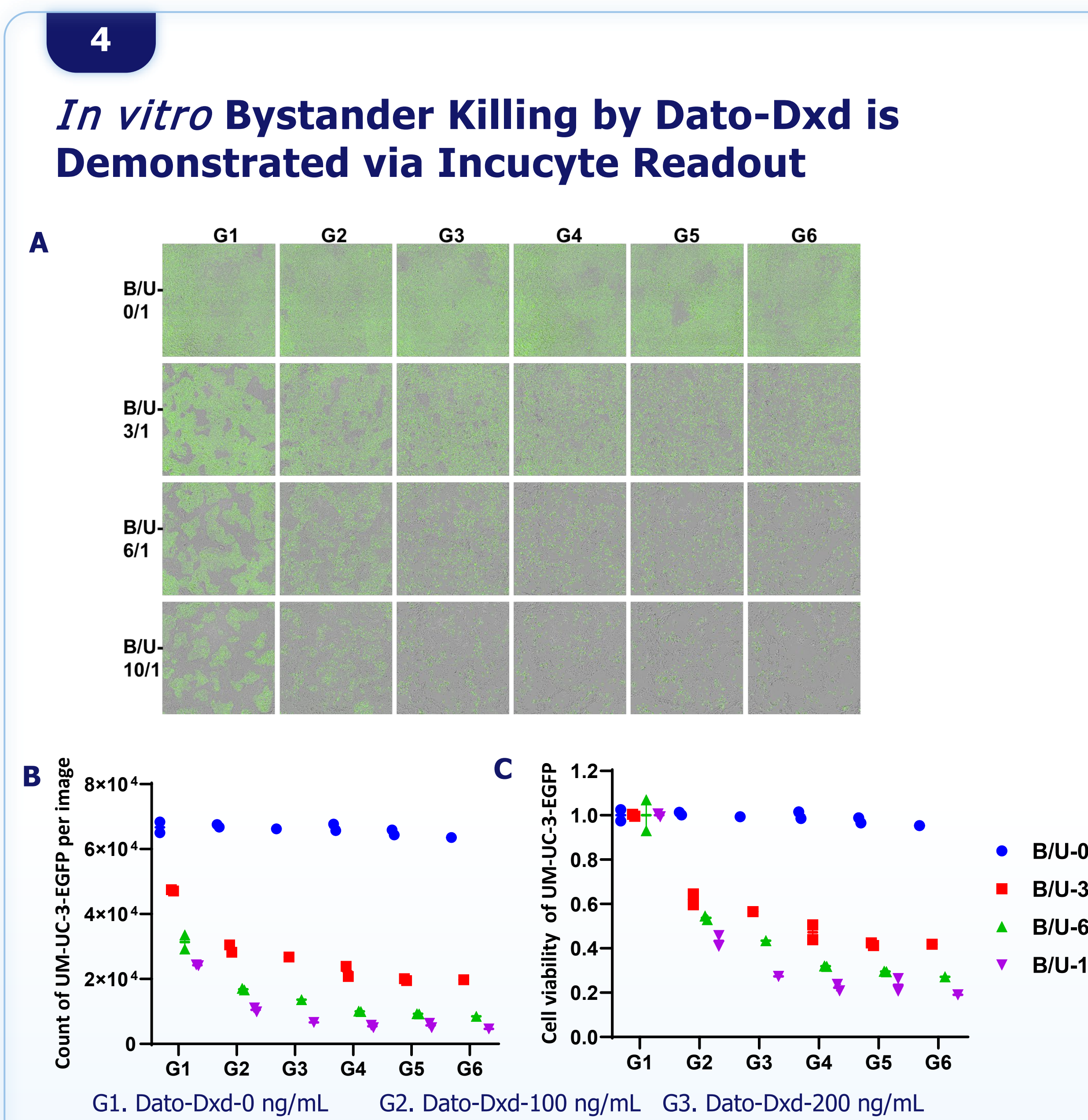
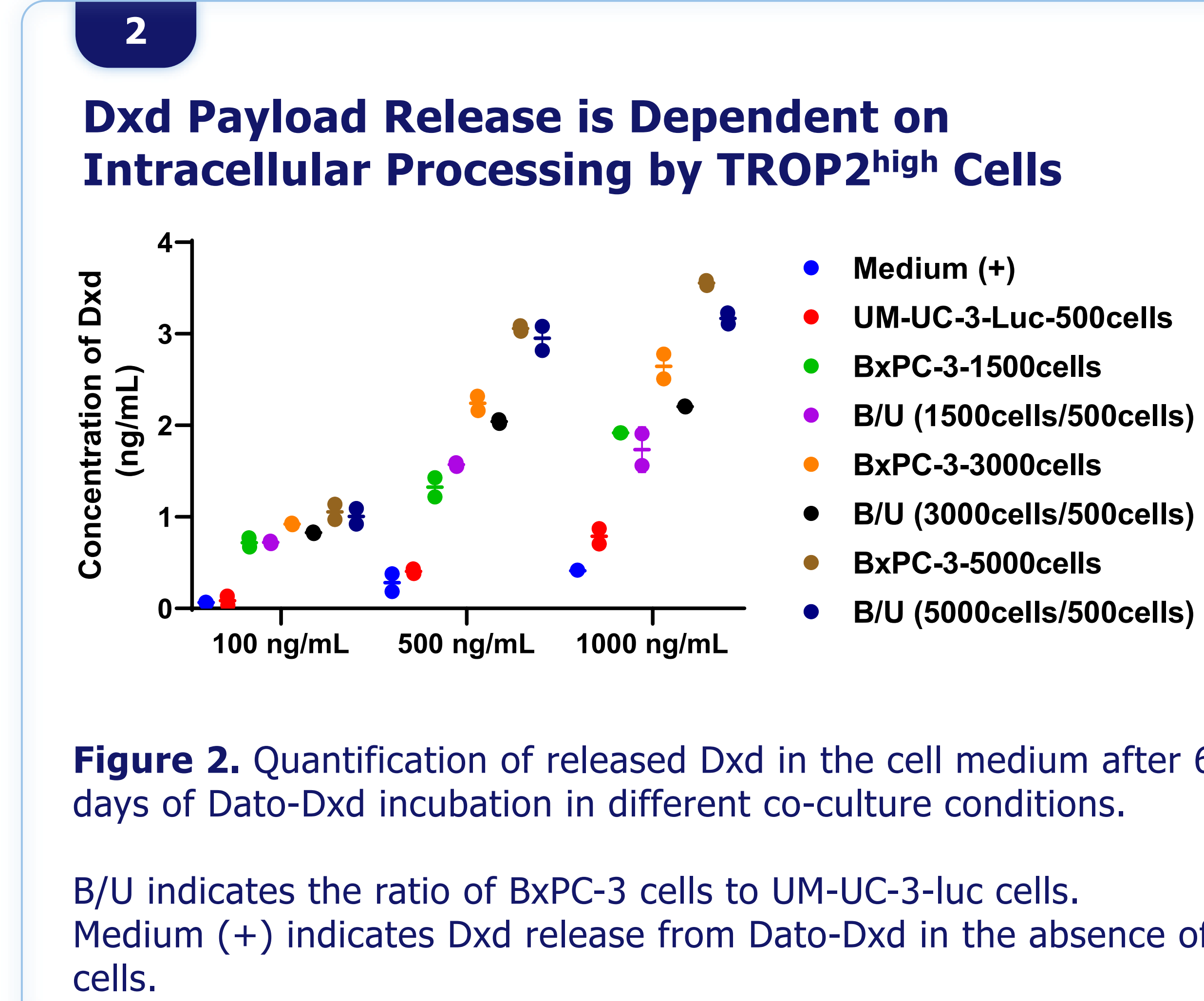
- The bystander killing effect of ADCs was assessed by both *in vitro* co-culture assays and *in vivo* mixed-cell-type xenograft tumor models.
- Four cell lines (NCI-N87, HCC1806, BxPC-3 and UM-UC-3) were selected based on their levels of TROP2 expression to evaluate the bystander killing effects.
- TROP2 expression was evaluated using Fluorescence-activated cell sorting (FACS), and the release of free payload was measured by liquid chromatography–mass spectrometry (LC–MS). Cell viability of TROP2^{low} cells in co-culture assays was quantified using the *In Vivo* Imaging System (IVIS) and Incucyte live-cell imaging.

Conclusion

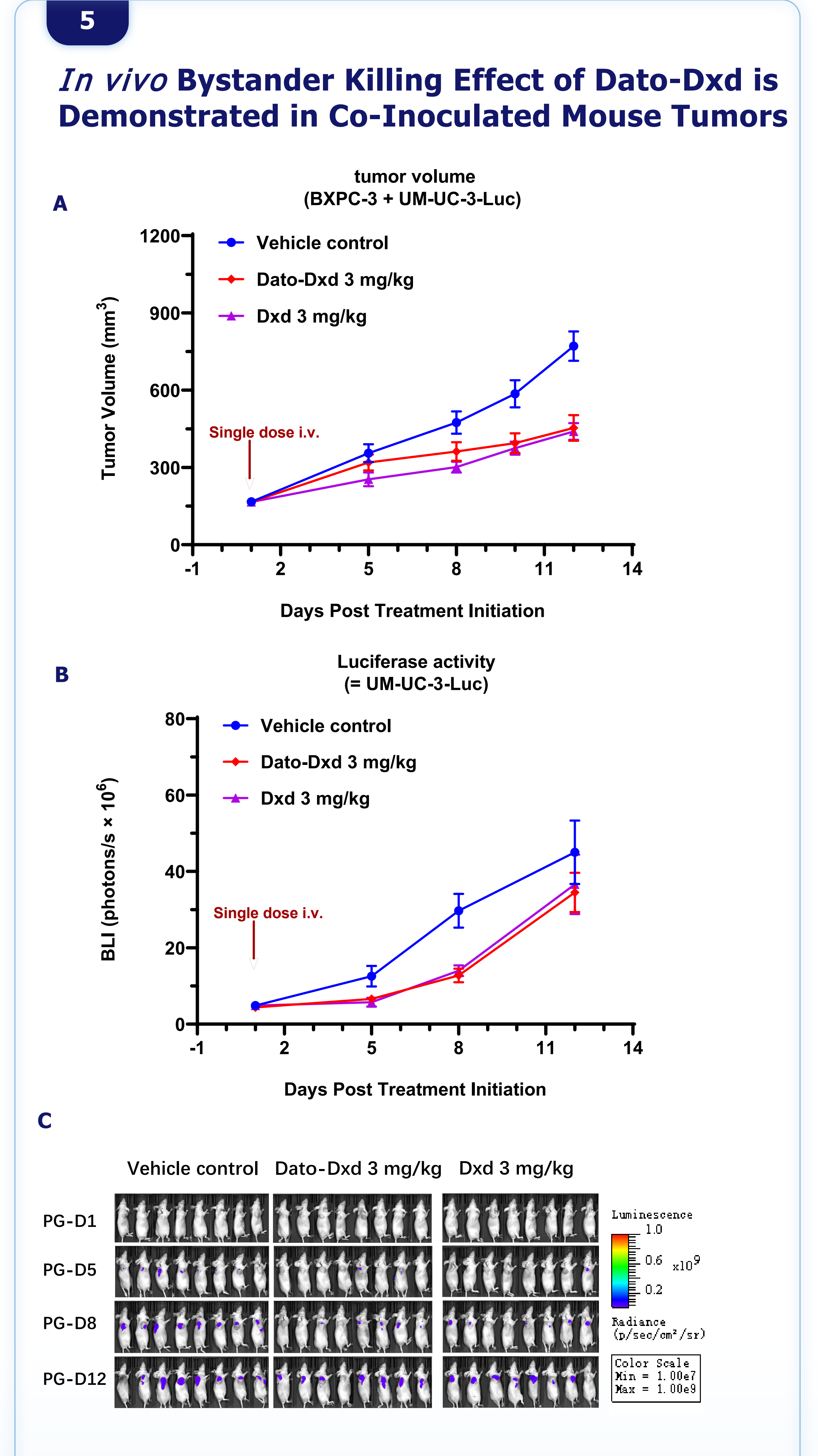
- Target-dependent cytotoxicity and intracellular Dxd payload release were verified to ensure the assay system reliably evaluates true bystander killing, excluding nonspecific effects from payload release due to unstable ADC.
- Both *in vitro* and *in vivo* assays are essential for fully understanding the key mechanism of bystander killing, verifying effects from simple cell culture systems to a complex living system, and ultimately predicting clinical success.



G1. Dato-Dxd-0 ng/mL G2. Dato-Dxd-100 ng/mL G3. Dato-Dxd-200 ng/mL
G4. Dato-Dxd-500 ng/mL G5. Dato-Dxd-1000 ng/mL G6. Dato-Dxd-2000 ng/mL



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A. Tumor growth curves of co-inoculated tumors (BxPC-3/UM-UC-3-luc) in BALB/c nude mice. B. Relative tumor volume of UM-UC-3-luc in BALB/c nude mice. C. Representative images of co-inoculated tumors (BxPC-3+UM-UC-3-luc) in BALB/c nude mice.