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Treatment of conjunctival melanoma cell lines with Bel-Sar induces immunogenic cell death

Sen Ma

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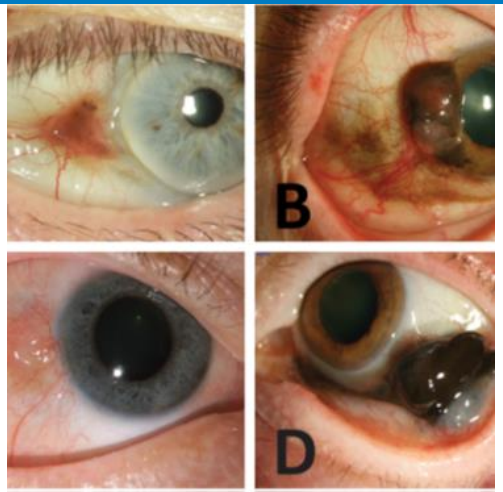

Disclosures

S. Ma, (F); **R.V. Huis in't Veld**, None; **E. de los Pinos**[a], (O); **F.A. Ossendorp**, None; **M.J. Jager** (F), None

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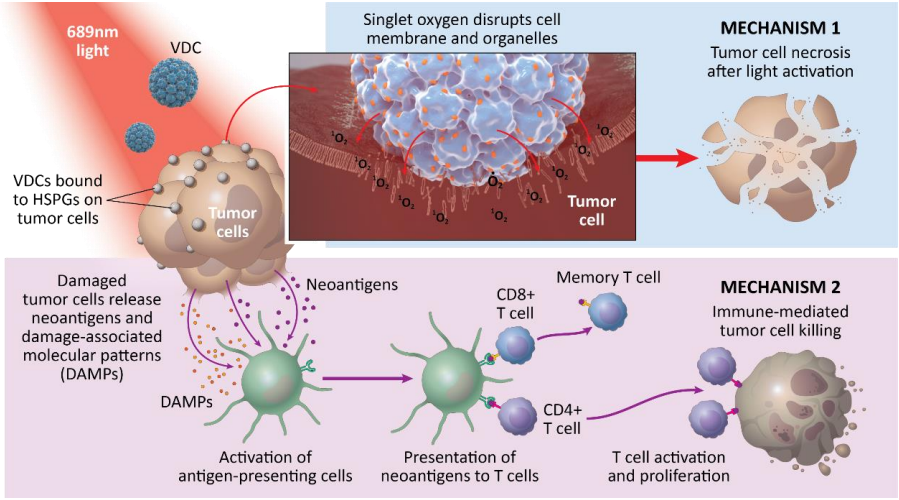
Bel-sar is a novel precision immunotherapy

- Bel-sar
  - Virus-like Drug Conjugate (VDC)
  - Ongoing Phase 3 randomized clinical trial in small choroidal melanoma and indeterminate lesions
- Bel-sar Mechanism of Action
  - Local (suprachoroidal) administration of bel-sar and activation with infrared laser
  - Reactive oxygen species (ROS), target tumor cell membrane,

    leading to:
    - Acute tumor cell necrosis
    - Immunogenic cell death (ICD)
    - CD4/CD8 T cell activation



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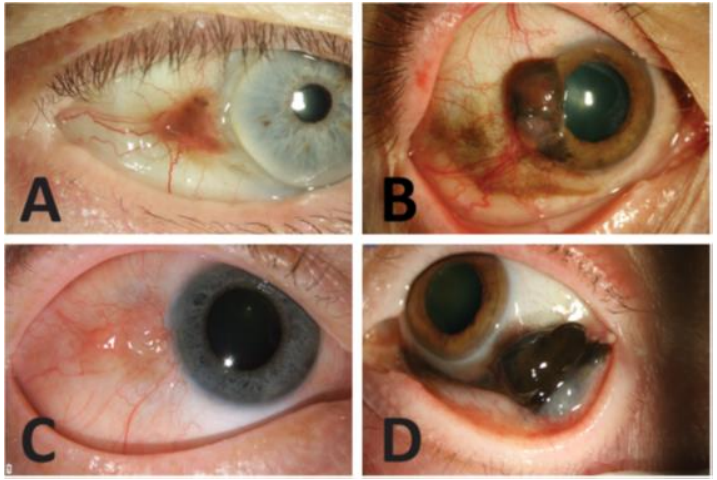
Bel-sar: prior works

1. Bel-sar specifically kills malignant tumor cells with a dual mechanism of action (Kines 2016)
2. Bel-sar has shown anti-tumoral activity in a rabbit orthotopic uveal melanoma model (Kines 2016)
3. Bel-sar: currently being investigated in a Phase 3 trial (NCT06007690) to treat indeterminate pigmented choroidal lesions and small melanomas
4. Bel-sar: also being investigated in a Phase 1 trial to treat bladder cancer (NCT05483868)
5. Mouse models show that the combination of Bel-sar with immune checkpoint inhibitors led to durable complete responses with an abscopal effect (Huis In't Veld 2023)

Conjunctival Melanoma (CJM)

- Conjunctival melanoma
  - Rare and malignant ocular tumor
  - BRAF and NRAS mutation
- Current treatment:
  - First line treatment for in situ CJM
    - Excision + cryotherapy to margins
    - Up to 50% local tumour recurrence
  - Metastasis
    - 26% cases
    - Kinase inhibitors, Immune checkpoint inhibitors:

      Currently, there are no drugs approved



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Research Questions

Can Bel-sar be used as an adjuvant to induce a stronger immune response to reduce recurrence rate or metastasis of CJM ?

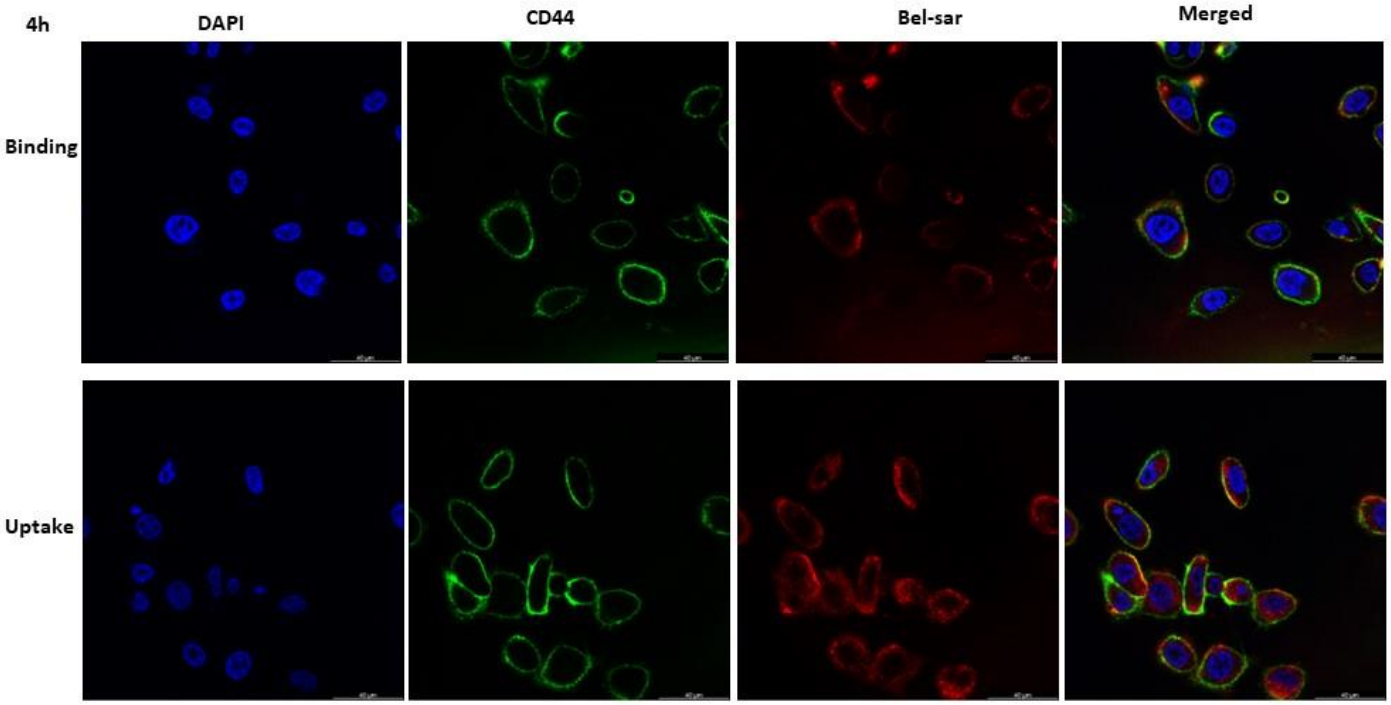
- Transmembrane transport process of Bel-sar
  - Explore intracellular and subcellular localization of Bel-sar at different time points
- Test Bel-sar treatment in CJM cell lines to assess:
  - Cytotoxicity
  - Immunogenic cell death
  - Immunostimulatory effect on Antigen Presenting Cells

Materials and methods

- CJM cell lines
  - Primary cell lines: CRMM1 (BRAF), CRMM2 (NRAS)
  - Recurrence cell line: CM2002.1 (BRAF)

- Analyses:
  - Intracellular localization: immunofluorescence microscope
  - Cytotoxicity: FACS, MTS assay
  - Damage associated molecular patterns (DAMPs): CRT, HSP90
  - Phagocytosis by macrophages: co-culture

Subcellular localization after addition to cells at 4° C for 4 hours : only membrane binding

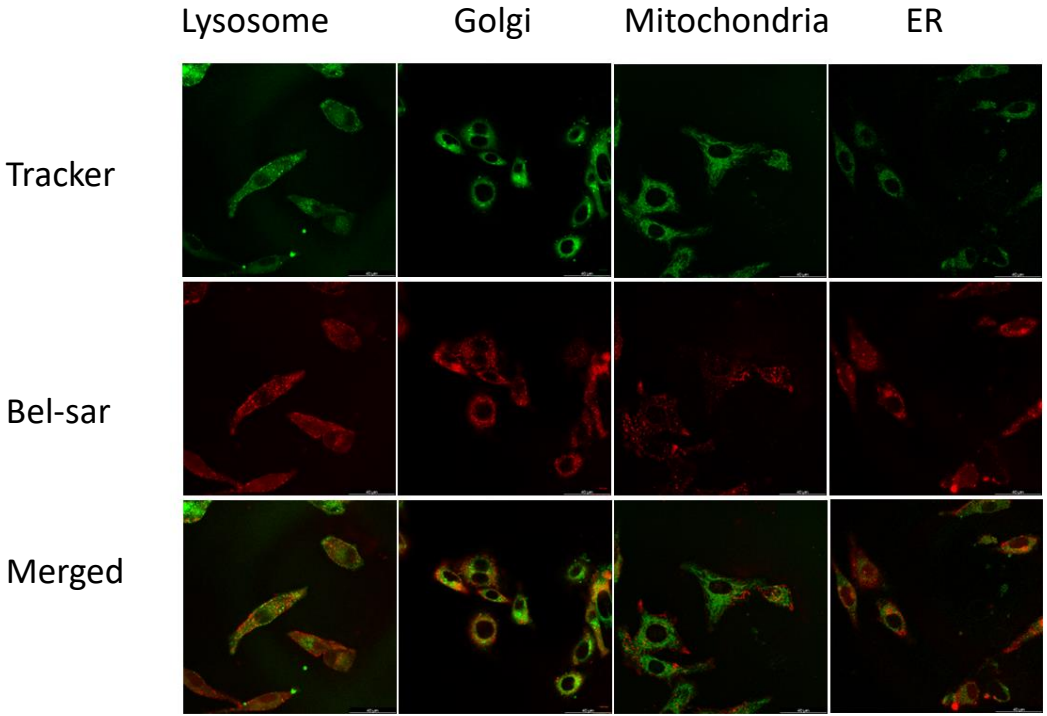


Binding at 4 °

Uptake at 37°

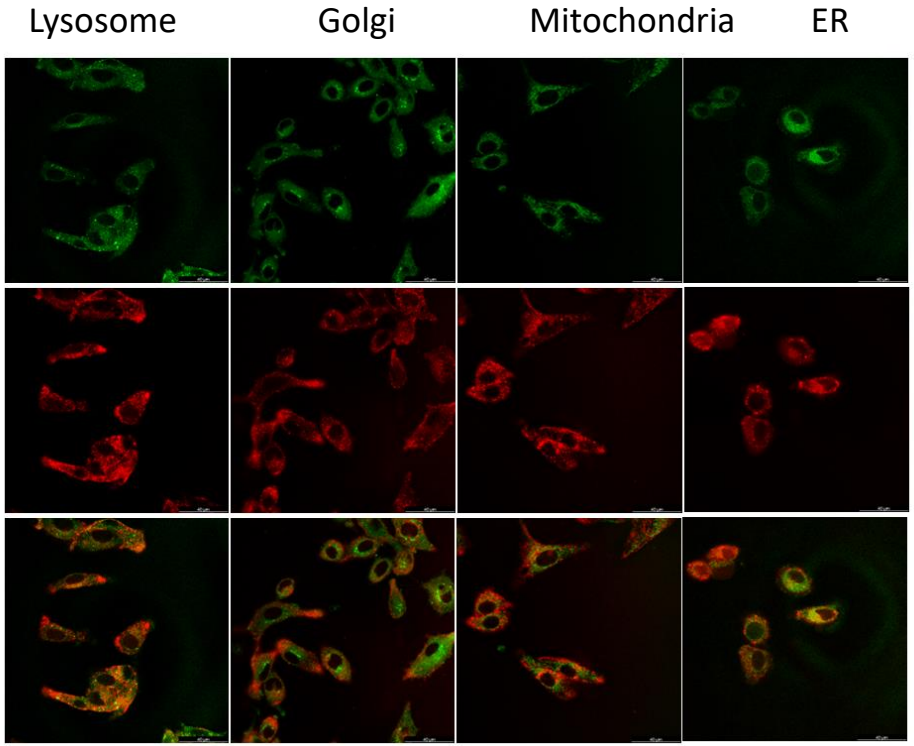
Bel-sar binds to the membrane, even at 4° C

Bel-sar intracellular localization: 4h at 37 ° C



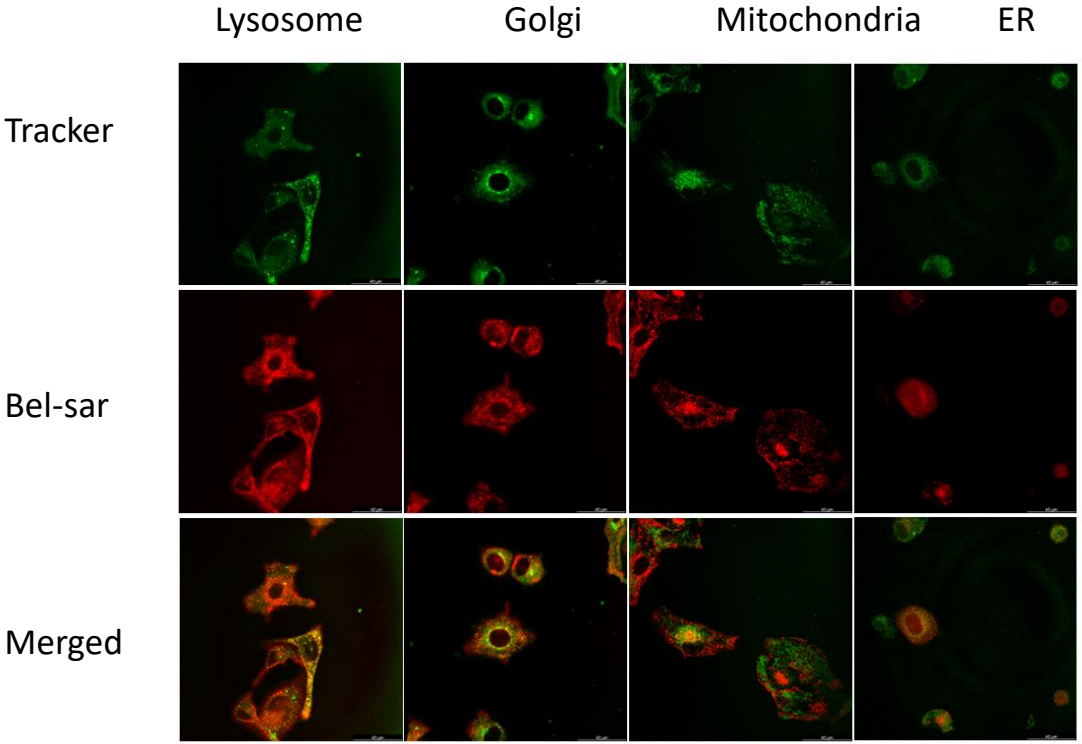
Bel-sar appears in the lysosomes, Golgi

Intracellular location 8 hours after addition to cells



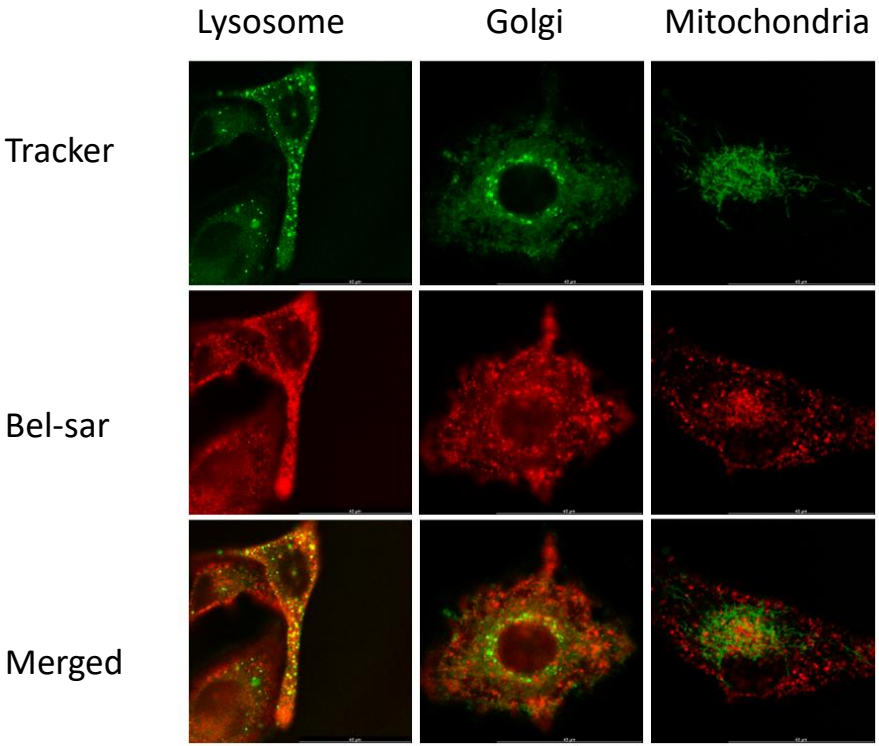
Bel-sar is inside lysosomes, Golgi

Intracellular localization of Bel-sar 24 hours after addition to cells



Bel-sar: mainly located in lysosome and partly in Golgi and Mitochondria

Intracellular localization of Bel-sar 24 hours after addition to cells, zoom in

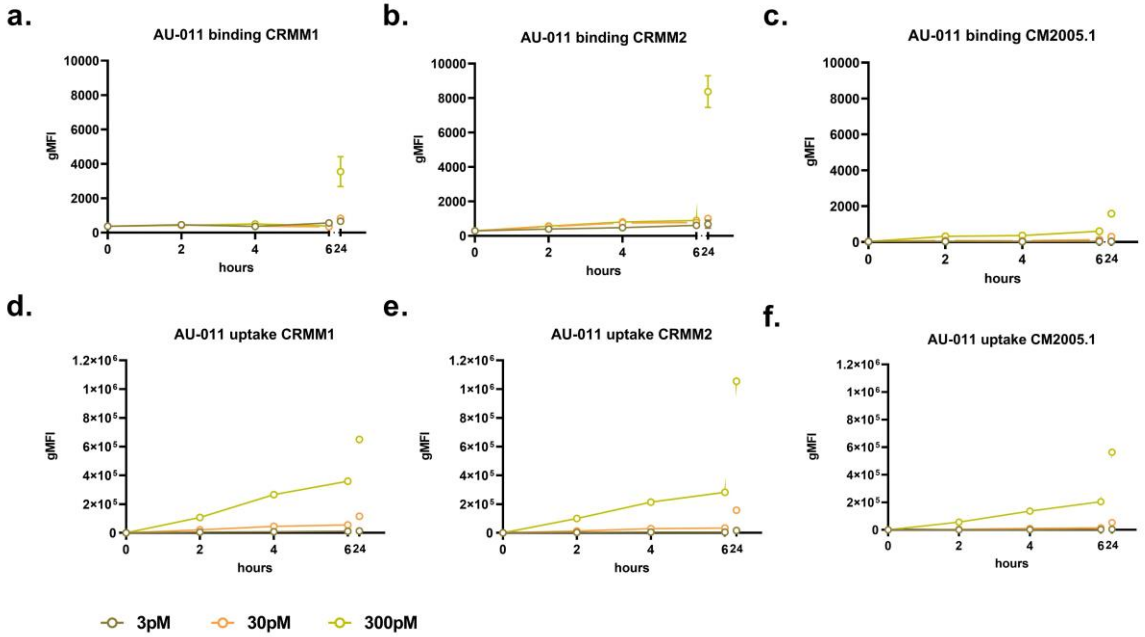


Bel-sar in lysosome, Golgi and mitochondria

Transmembrane Process of Bel-sar

	Membrane	Cytoplasm	Lysosome	Golgi	Mitochondria	ER
4h at 4 °C	+	-	-	-	-	-
4h at 37 °C	+	+	+	-	-	-
8h	+	+	+	-	-	-
24h	+	+	+	+	+	-

Binding of Bel-sar at 4 °C and 37 °C

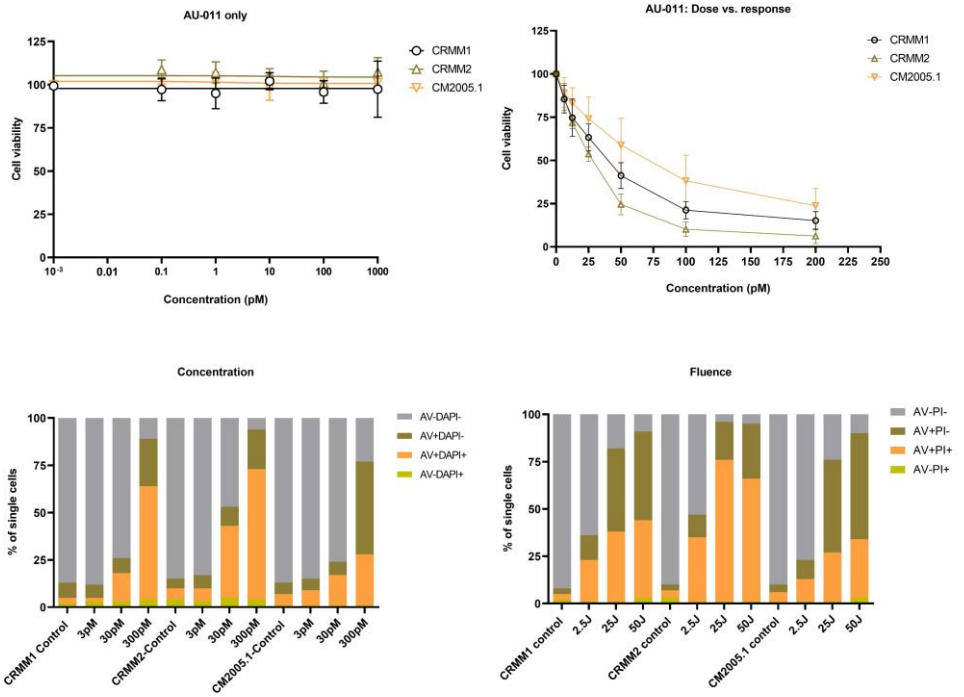


Binding at 4 °

Uptake at 37°

Bel-sar binding and uptake increased over time and with concentration.
At 37 °C (uptake), the gMFI was significantly higher than at 4 °C (binding).

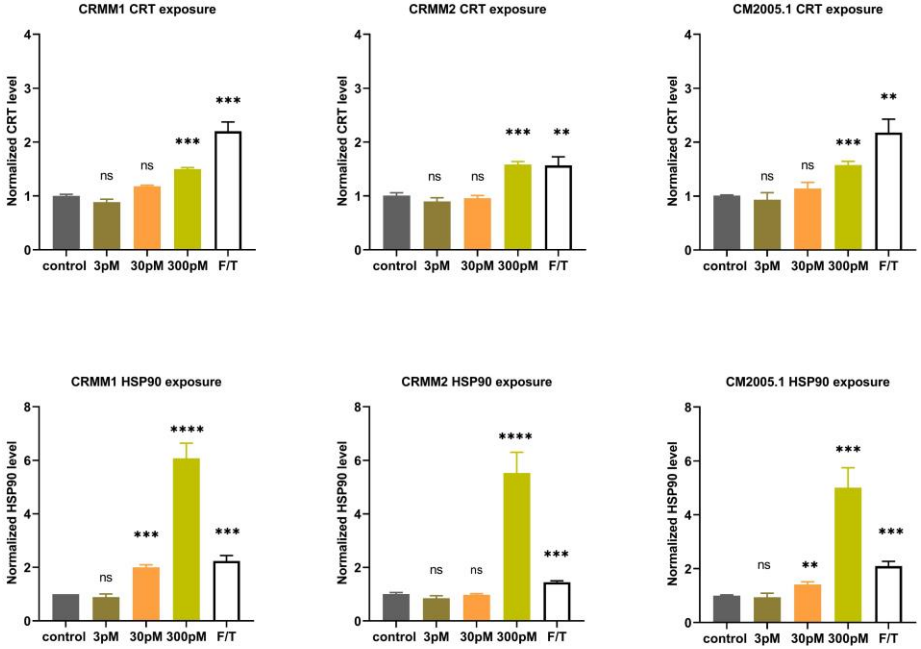
Cell Viability and Cytotoxicity



IC50
CRMM1: 40 pM
CRMM2: 31 pM
CM2005.1: 60 pM

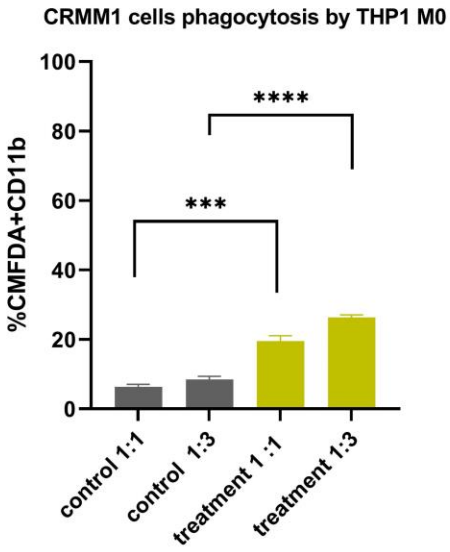
Bel-sar induced cell death in a concentration and fluence dependent manner.

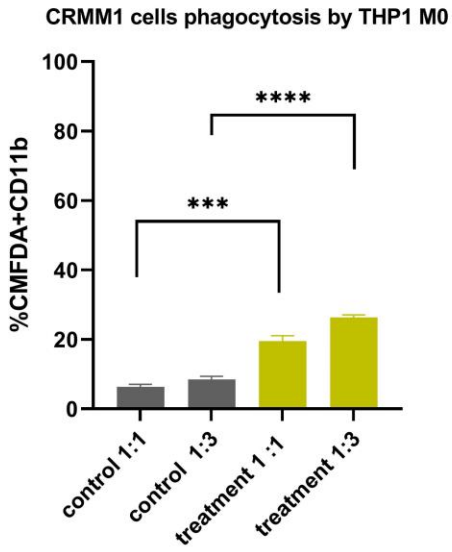
Exposure of Damage Associated Molecular Patterns (DAMPs), Calreticulin (CRT) and HSP90 after Treatment with Bel-sar

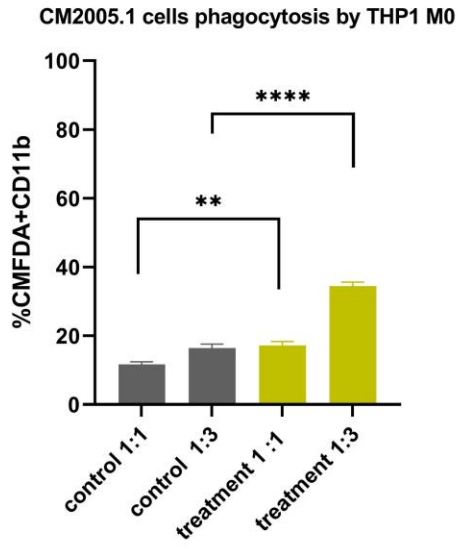


Bel-sar enhanced the membrane exposure of CRT and HSP90

Phagocytosis by THP-1 Derived Macrophages after treatment with Bel-sar







Bel-sar enhanced the phagocytosis of tumor cells by macrophages

Conclusions

1. Bel-sar binding was observed in all three CJM cell lines tested including primary and recurrent cell lines.

2. Bel-sar is distributed to lysosomes, Golgi and mitochondria.

3. Bel-sar induced effective cell death in the three CJM cell lines tested.

4. Bel-sar induced immunogenic cell death, characterized by enhanced exposure of DAMPs and engulfment by THP1-derived macrophages.

Discussion and Next Steps

- Can Bel-sar induce other types of cell death?
  - Ferroptosis (lysosome related)
  - Pyroptosis (Golgi, mitochondria)

- Explore clinical development of Bel-sar in ocular surface and eyelid malignancies

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Thank you

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