Novel triple-drug cocktail for glioblastoma exhibits potent synergistic antitumoral effects

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CRS 2025 ANNUAL MEETING & EXPOSITION
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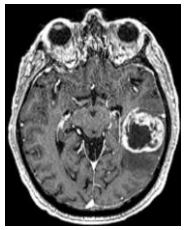
NEXT-GENERATION DELIVERY INNOVATIONS

The burden of Glioblastoma (GBM)

- Most **common** and **lethal** type of brain cancer
- **15 months median survival** and very high **recurrence** rate (~90%)



- Standard-of-care → Surgery + Radiotherapy + **Chemotherapy with Temozolomide (TMZ)**

Stupp et al., N. Engl. J. Med, 2005

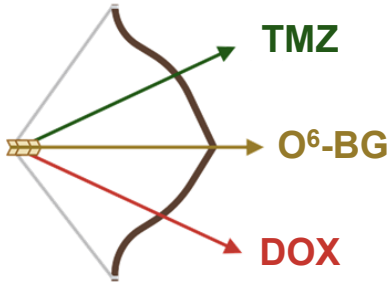
- **Chemoresistance and Blood-brain barrier (BBB)** pose main challenges for treatment

MGMT

OUR SOLUTION

Triple drug cocktail in hydrogel to defeat GBM

- **Eradicate** tumor cells with **TMZ**
- **Revert chemoresistance** to **TMZ** with **O^6-Benzylguanine**
- **Trigger anti-tumoral immune responses** with **Doxorubicin**

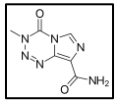


OUR GOAL

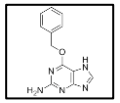
Stop tumor recurrence while reducing side effects

Drug cytotoxicity in 2D GBM models

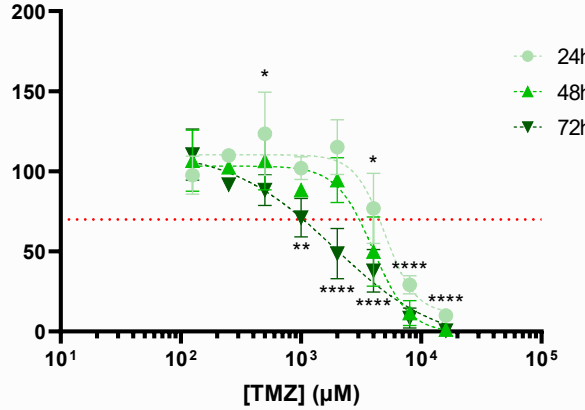
Temozolomide (TMZ)

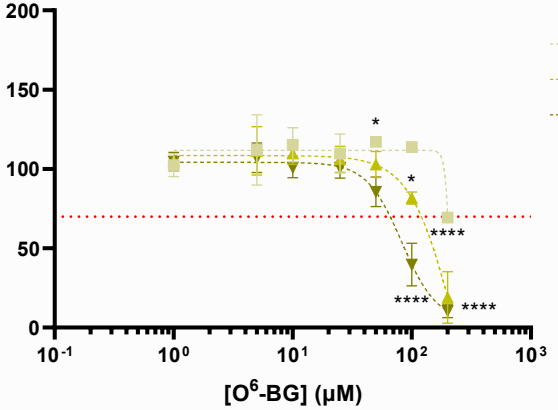


O⁶-Benzylguanine (O⁶-BG)

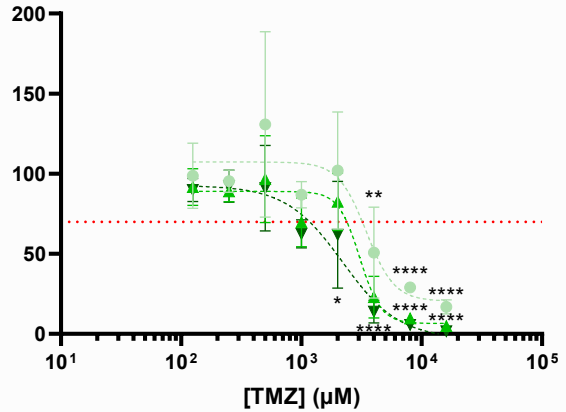


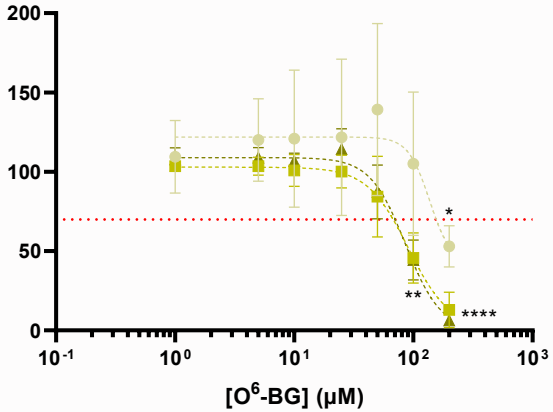
T98G





U87





IC_{50} TMZ (72h)
- **T98G:** 1871 μM
- **U87:** 2198 μM

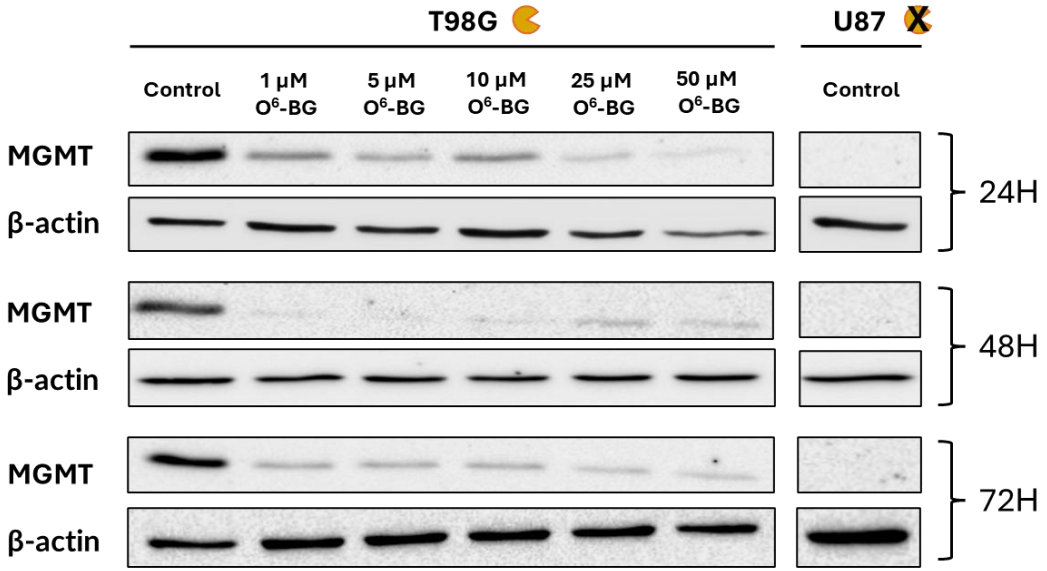
IC_{50} O^6-BG (72h)
- **T98G:** 82 μM
- **U87:** 86 μM

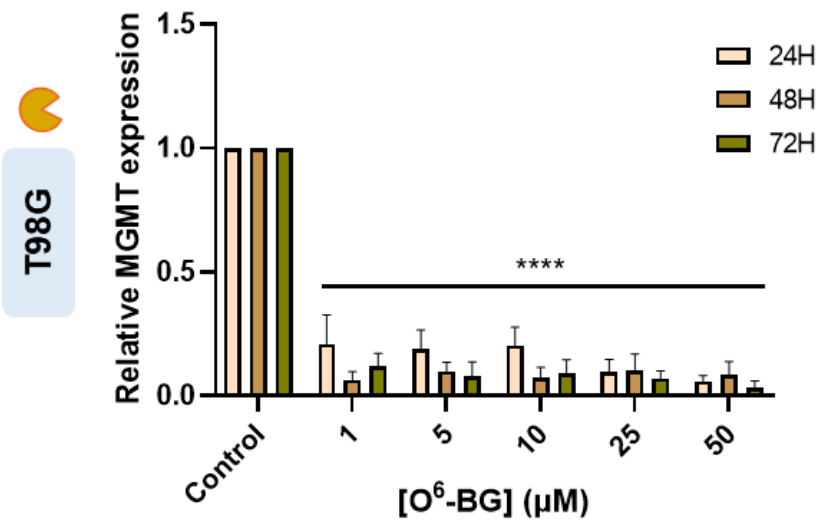
Can O^6-BG synergize with TMZ and sensitize cells to this chemotherapy?

*p <0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 relative to untreated cells – control

O^6-BG suppresses MGMT expression in GBM 2D models

2D GBM cell monocultures





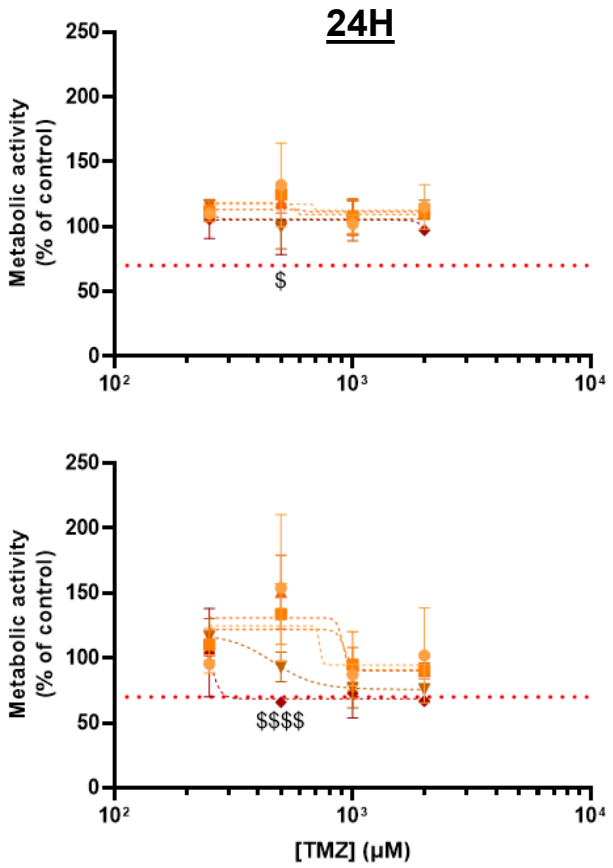
Long-lasting MGMT inhibition by O^6-BG reverts chemoresistance to TMZ and sensitizes GBM cells to chemotherapy

****p < 0.0001 relative to control – untreated cells

TMZ and O^6-BG synergism in 2D GBM models

T98G

U87



TMZ + O^6-BG defeat GBM 2D models

↑ **Cytotoxicity** ($$$$)

↑ **Synergism** (CI<1)

(Chou-Talalay analysis)

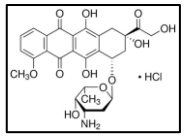
$p < 0.05; $^{$$}p < 0.01; $^{$$$}p < 0.001; $^{$$$$}p < 0.0001 relative to TMZ monotherapy; CI – combination index

Drug cytotoxicity in 2D GBM models

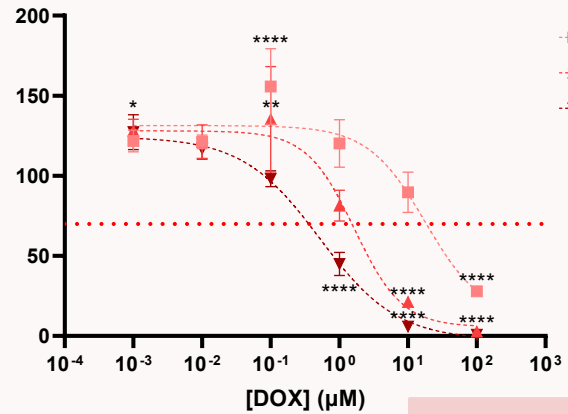
Temozolomide (TMZ)

O⁶-Benzylguanine (O⁶-BG)

Doxorubicin (DOX·HCl)



T98G



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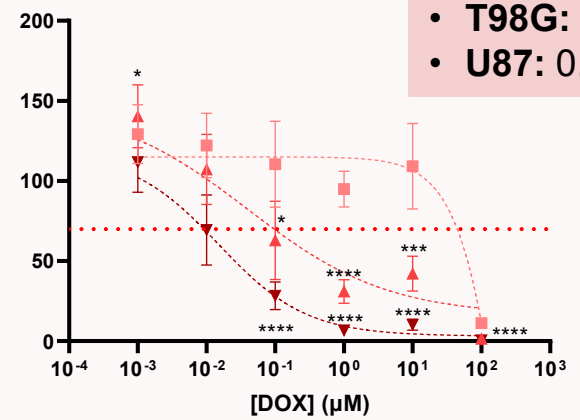


IC_{50} DOX (72h)

- **T98G:** 0.52 μM
- **U87:** 0.02 μM

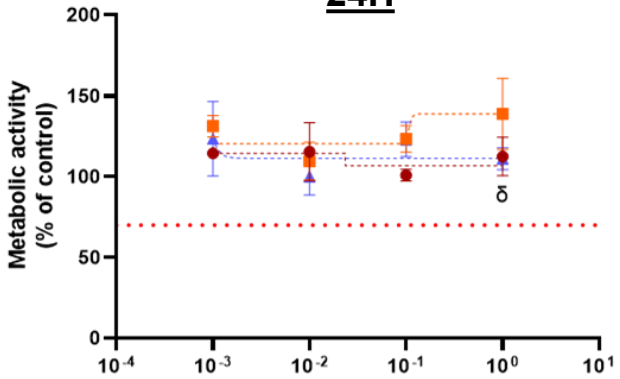
*p <0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 relative to untreated cells – control

TOX cocktail synergism in 2D GBM models

TRIPLE drug cocktail → **T**MZ + **O**^{6}-BG + DO**X** = **TOX**

24H

T98G



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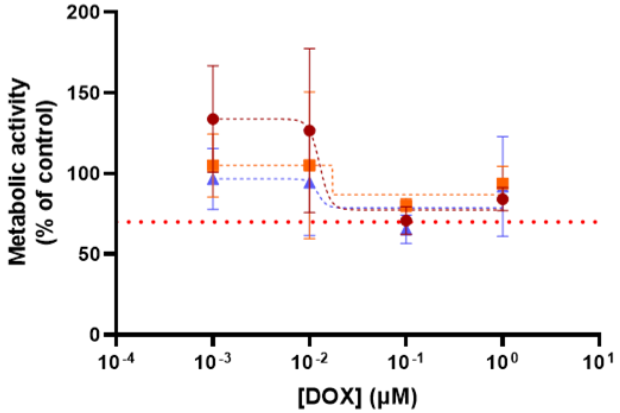


TOX cocktail defeats 2D GBM models

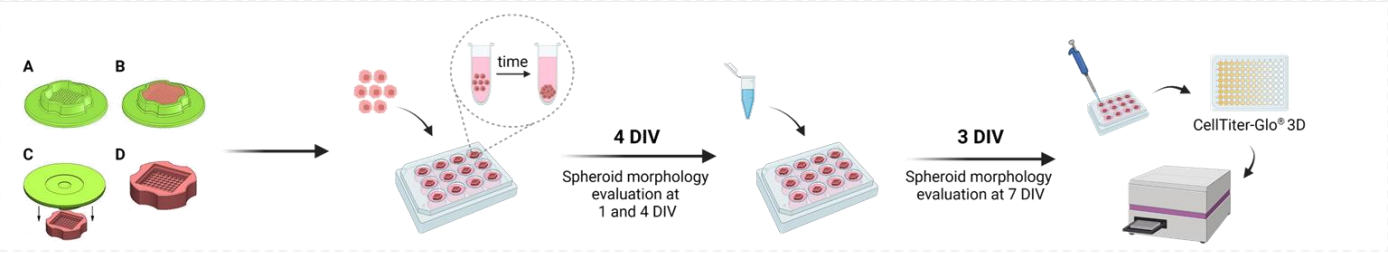
↑ **Cytotoxicity** (δδ)

↑ **Synergism** (CI<1)

(Chou-Talalay analysis)

$^{***}p < 0.001$; $^{****}p < 0.0001$ relative to cells treated with DOX; $^{\delta}p < 0.05$; $^{\delta\delta}p < 0.01$ relative to cells without O^6-BG; CI – combination index

TOX cocktail synergism in 3D GBM spheroid models

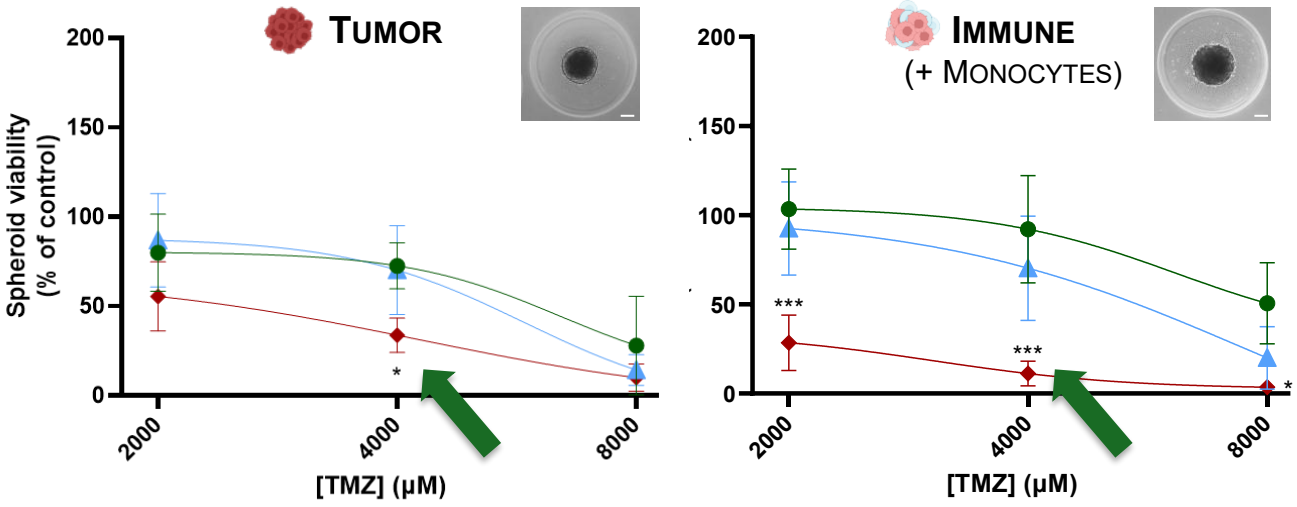


Martins et al., JCR, 2023

TOX cocktail destroys 3D tumor and immune GBM spheroids

- ↑ **Cytotoxicity**
- ↑ **Synergism** (CI<1) (Chou-Talalay analysis)

T98G



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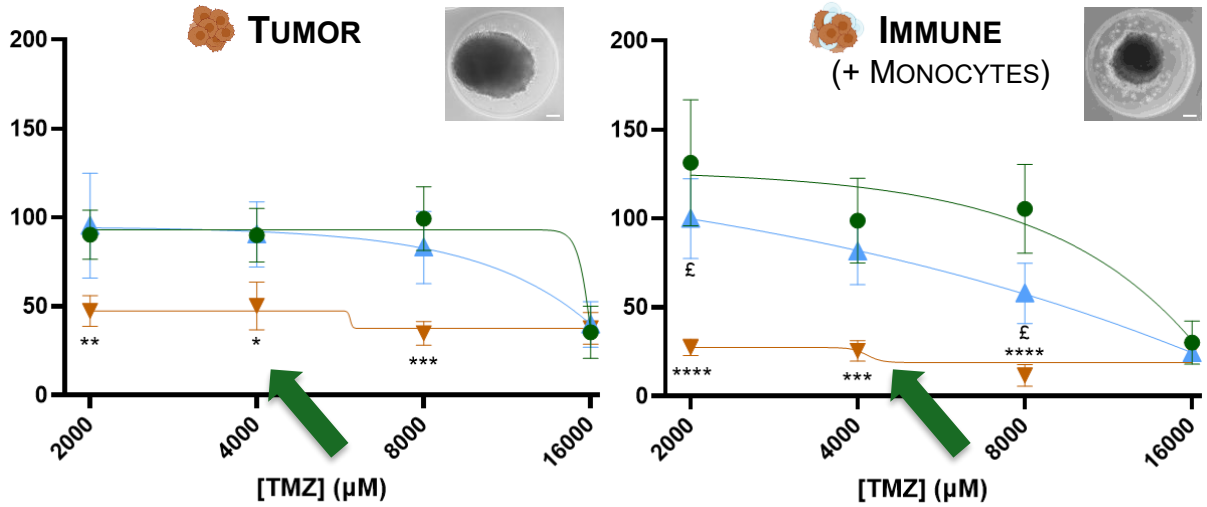
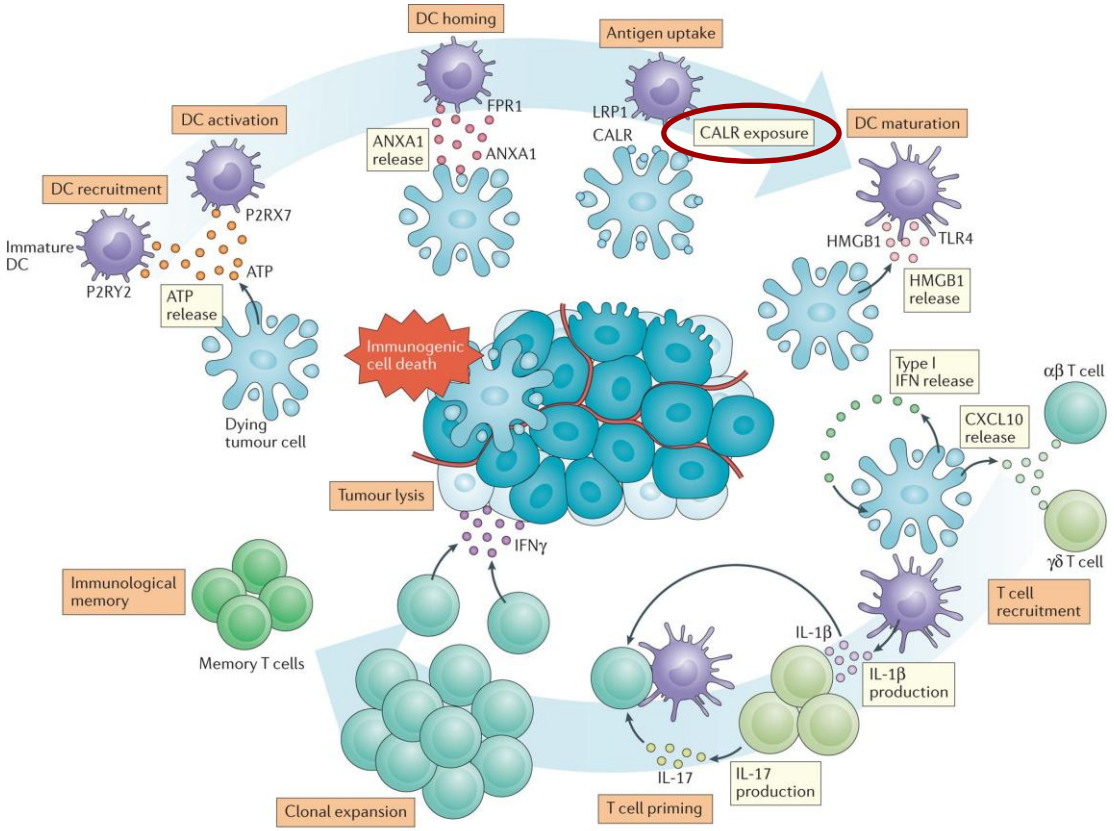


TMZ | TMZ + O^6-BG 50 μM | TOX 1 | TOX 7

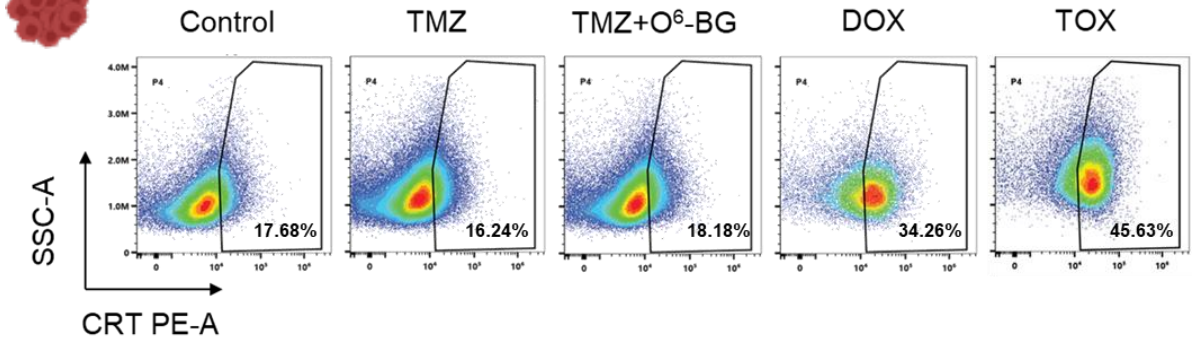
*p < 0.05; ***p < 0.001; ****p < 0.0001 relative to untreated cells – control; £p < 0.05 relative to TMZ monotherapy; Scale bar = 100 μm; CI – combination index

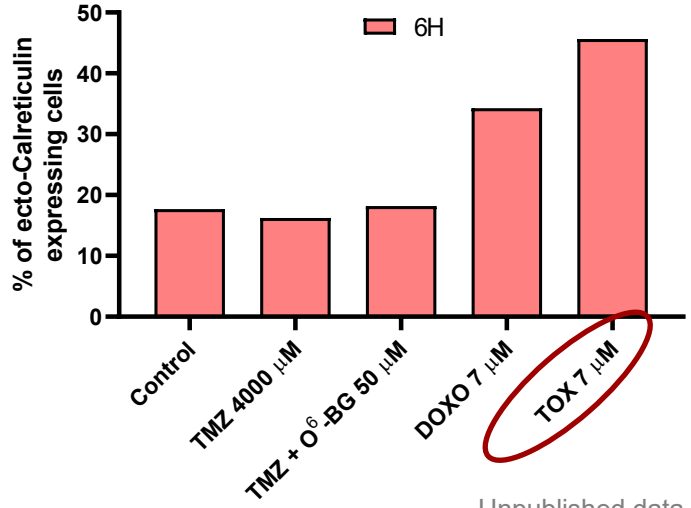
TOX cocktail stimulates calreticulin translocation in 3D GBM spheroid models



Galluzzi et al., Nat Rev Immunol, 2017

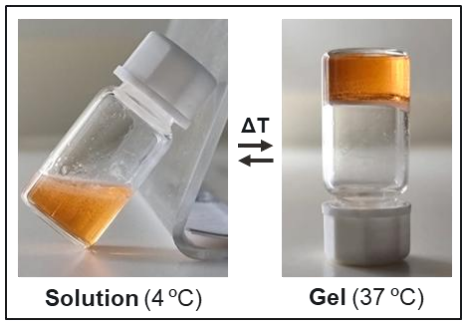
T98G





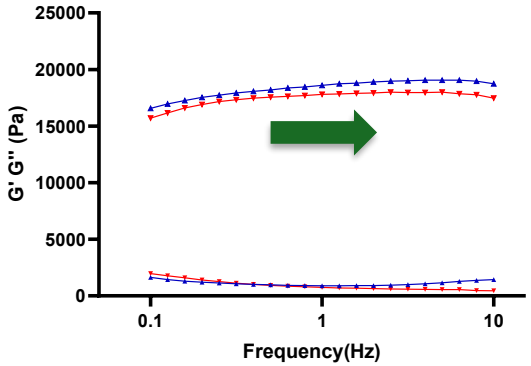
Unpublished data, please do not share

TOX-loaded hydrogel for GBM *in situ* therapy

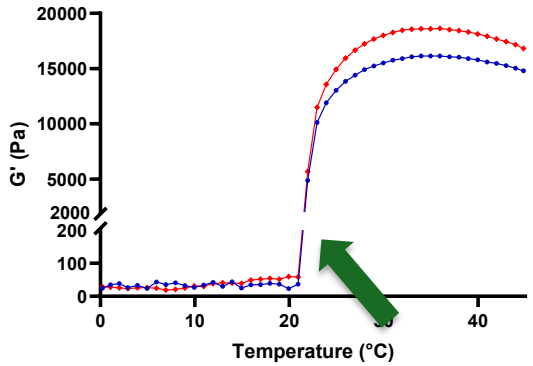


TOX-LOADED HYDROGEL
made of 25 wt% Pluronic F127
+ 2 wt% Hyaluronic Acid

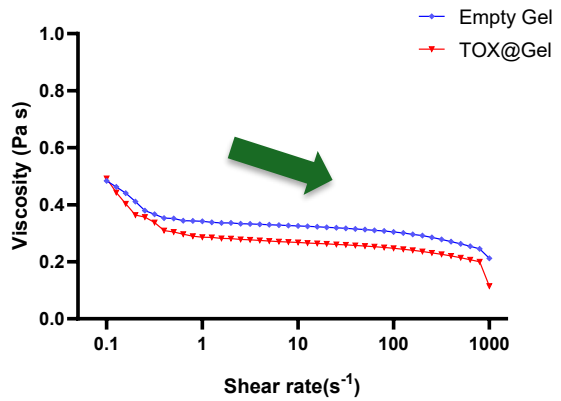
- **Frequency sweep**



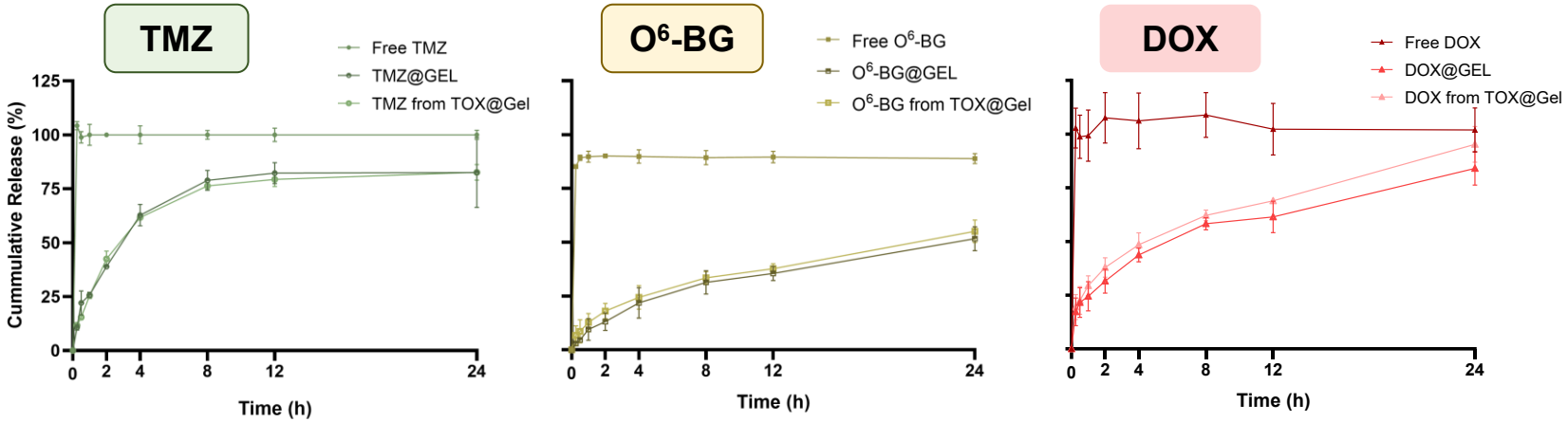
- **Sol-gel transition**



- **Viscosity**

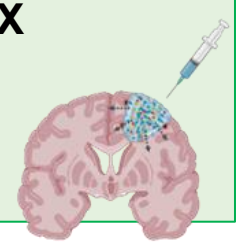


Drug release from hydrogels *in vitro* (aCSF, pH 6.5)



TOX@PF-HA hydrogels are ideal for local GBM therapy

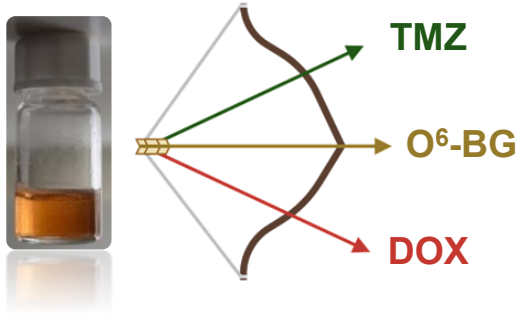
- ✓ **Sustained release of TOX**
- ✓ **Gel-like** behavior
- ✓ **Thermo-responsiveness**
- ✓ Good **injectability**



Unpublished data, please do not share

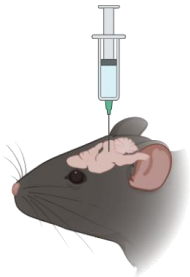
Main Conclusions

- **Strong** and **synergistic** anti-tumoral action of **TOX** against 2D and 3D GBM models
- **MGMT suppression** by O^6-BG is key to **sensitize cells to TMZ**
- **DOX** boosts the **potency** of the combinatorial treatment
- Successful preparation of **TOX-loaded hydrogel** for **localized** GBM treatment



Future steps

- Evaluate the therapeutic efficacy of **TOX-loaded hydrogel *in vivo***



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