Astrocyte “Cellular Backpacks” for Targeted Drug Delivery to Melanoma Brain Metastases via Innate Migration

11/29/2023 BME 771 Final Project Presentation

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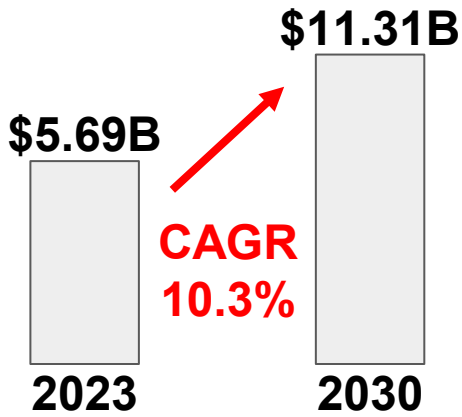
MBM exhibits high prevalence & mortality

- Melanoma represents **1%** skin cancer cases but **80%** skin cancer-related death due to high metastatic potential
- 20-40% metastatic melanoma patients diagnosed with & 50% later develop brain metastasis (MBM)
- MBM patients' median survival = **12.8 month** with mortality rate = **80-85%**
- Likely to grow with increasing UV exposure since 50% cutaneous melanoma will develop MBM



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Tan, X. L. et al. Burden and Risk Factors of Brain Metastases in Melanoma: A Systematic Literature Review. Cancers (Basel) 14 (2022)
Vosoughi, E. et al. Survival and clinical outcomes of patients with melanoma brain metastasis in the era of checkpoint inhibitors and targeted therapies. BMC Cancer 18, 490 (2018)
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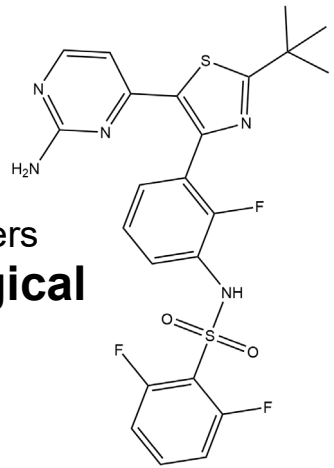
Research, V. M. Global Drugs For Melanoma Market Size By Test (Chemotherapy, Immunotherapy, Targeted Therapy), By Application (Hospitals, Clinics), By Geographic Scope And Forecast. Verified Market Research (2023).

Current MBM treatments face clinical challenges

- 50% cutaneous melanoma & 90% MBM carry BRAF mutation resulting in 100-200 times more active BRAF
- BRAFi, such as **dabrafenib**, shows high efficacy in reducing melanoma tumors size & used for treating MBM through oral administration
- **Challenges**
  - Limited penetration through blood-brain barrier (BBB)
  - Limited delivery around or into tumor
  - Serious adverse effects: cerebral hemorrhage & neurotoxicity
  - Rapid clearance due to being dual substrate of BCRP & P-gp efflux transporters
- Sub-pharmacological concentration makes brain a **pharmacological sanctuary**, limiting duration of response
  - Promote drug resistance & relapses
  - ↑ oral dose can lead to additional systemic adverse effects



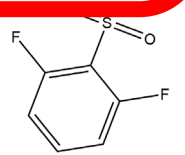
Saberian, C., Sperduto, P. & Davies, M. A. Targeted therapy strategies for melanoma brain metastasis. Neurooncol Adv 3, v75-v85 (2021)
Mittapalli, R. K., Vaidhyanathan, S., Dudek, A. Z. & Elmquist, W. F. Mechanisms limiting distribution of the threonine-protein kinase B-RaF(V600E) inhibitor dabrafenib to the brain: implications for the treatment of melanoma brain metastases. J Pharmacol Exp Ther 344, 655-664 (2013)Phadke, M., Ozgun, A., Eroglu, Z. & Smalley, K. S. M. Melanoma brain metastases: Biological basis and novel therapeutic strategies. Exp Dermatol 31, 31-42 (2022)
MedlinePlus. Dabrafenib: MedlinePlus Drug Information. Medlineplus.gov (2023).

Current MBM treatments face clinical challenges

- 50% cutaneous melanoma & 90% MBM carry BRAF mutation resulting in 100-200 times more active BRAF
- BRAFi, such as **dabrafenib**, shows high efficacy in reducing melanoma

A more effective delivery strategy of dabrafenib is needed for treating MBM with less adverse effects

sanctuary, limiting duration of response
  - Promote drug resistance & relapses
  - ↑ oral dose can lead to additional systemic adverse effects



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MedlinePlus. Dabrafenib: MedlinePlus Drug Information. Medlineplus.gov (2023).

MBM-facilitating astrocytes provide novel delivery pathway

- Astrocytes responsible for repairing neurons after brain injuries & maintain basic pH of cerebrospinal fluid (CSF)
- Interaction with astrocytes increases melanoma cells aggressiveness, proliferative potentials & chemotherapy resistance
  - Early MBM: melanoma cells recruit & directly contact astrocytes through gap junctions
  - Late MBM: astrocytes infiltrate tumor
- Recruitment, proximity & intratumor migration make astrocytes highly favorable for innovative drug delivery

Lee, H. G., Wheeler, M. A. & Quintana, F. J. Function and therapeutic value of astrocytes in neurological diseases. Nat Rev Drug Discov 21, 339-358 (2022)
Klein, A. et al. Astrocytes facilitate melanoma brain metastasis via secretion of IL-23. J Pathol 236, 116-127 (2015)
Lin, Q. et al. Reactive astrocytes protect melanoma cells from chemotherapy by sequestering intracellular calcium through gap junction communication channels. Neoplasia 12, 748-754 (2010)
Wasilewski, D., Priego, N., Fustero-Torre, C. & Valiente, M. Reactive Astrocytes in Brain Metastasis. Front Oncol 7, 298 (2017)

Strategy tailored to astrocyte-melanoma cell interactions

- Astrocytes recruited by melanoma cells & infiltrate tumor
- Astrocyte-melanoma cell reciprocal stimulation promotes melanoma cells' MMP-2 secretion

- Astrocytes as cellular backpack to carry dabrafenib to proximity of melanoma cells & tumor
- Use MMP-2 degradable peptide chain to link dabrafenib to astrocytes

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- Astrocytes maintain basic pH in CSF
- Melanoma cells create acidic tumor environment (TME)

→

- Acid-labile microgel that encapsulates cellular backpacks to minimize damages during injection

Strategy tailored to astrocyte-melanoma cell interactions

Aim 1

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- Astrocyte-melanoma cell reciprocal stimulation promotes melanoma cells' MMP-2 secretion

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- Astrocytes as cellular backpack to carry dabrafenib to proximity of melanoma cells & tumor
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Aim 2

- Astrocytes maintain basic pH in CSF
- Melanoma cells create acidic tumor environment (TME)

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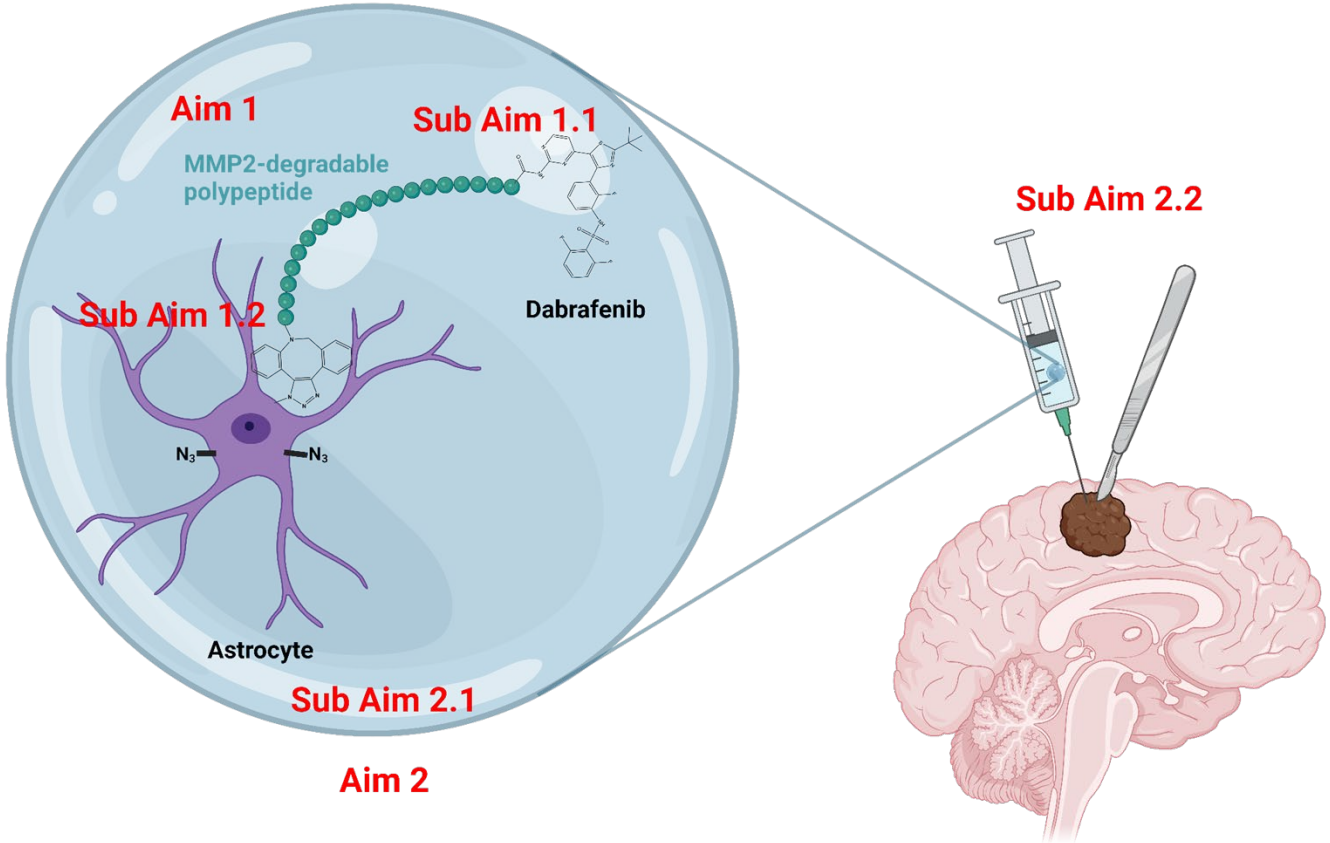
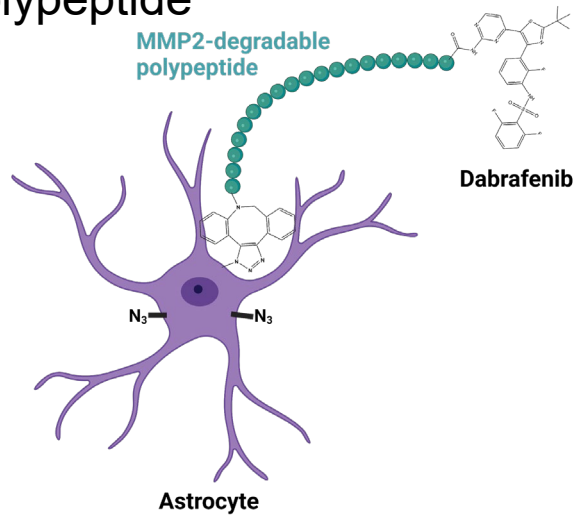
- Acid-labile microgel that encapsulates cellular backpacks to minimize damages during injection & for structural support

Microgel-protected astrocyte backpack to treat MBM



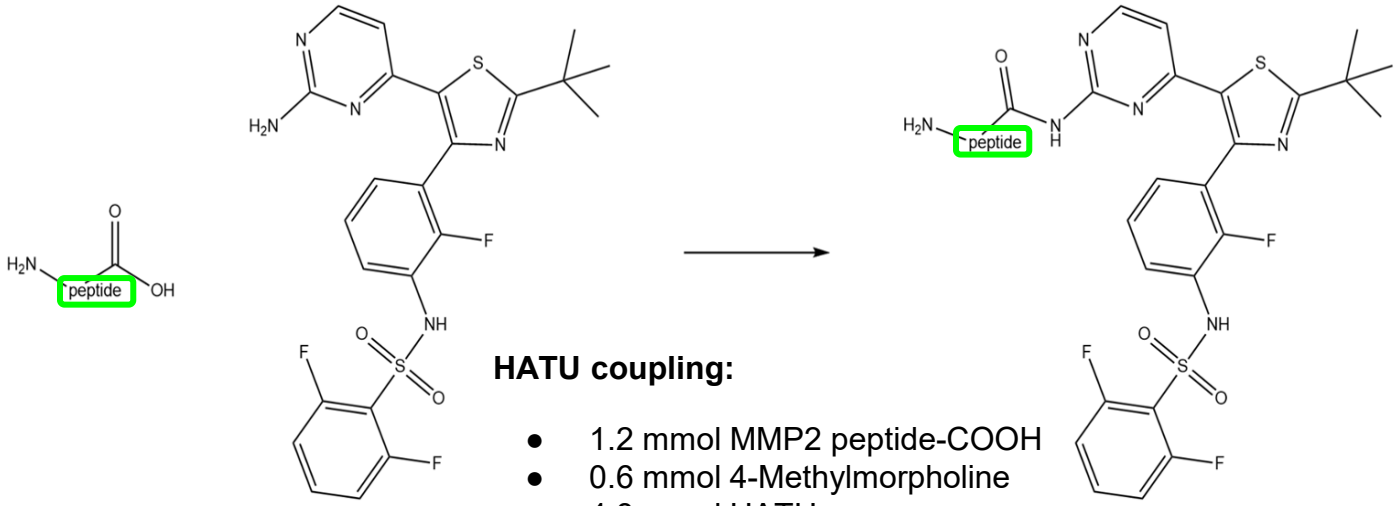
Aim 1 Engineer patient-derived astrocytes as dabrafenib-carrying “cellular backpacks”

- Sub aim 1.1 Conjugate dabrafenib with MMP-2-degradable polypeptide
- Sub aim 1.2 Conjugate patient-derived astrocytes with dabrafenib-loaded MMP-2-degradable polypeptide



Aim 1.1 Conjugate dabrafenib with polypeptide

Conjugating C-terminus of MMP-2 degradable peptide with free amine of dabrafenib

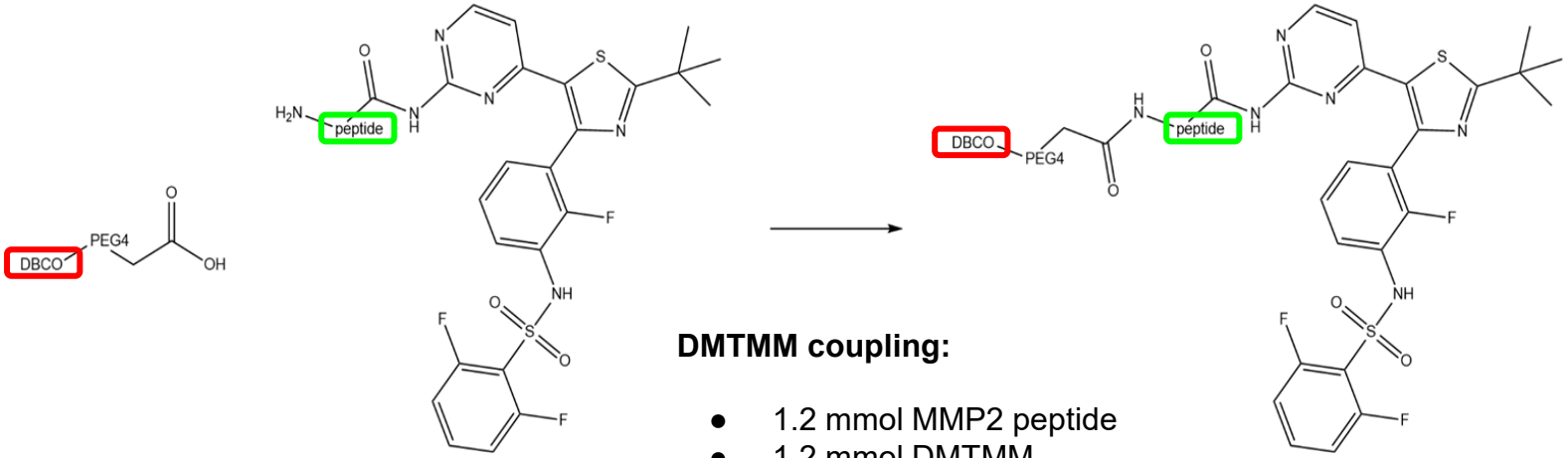


HATU coupling:

- 1.2 mmol MMP2 peptide-COOH
- 0.6 mmol 4-Methylmorpholine
- 4.8 mmol HATU
- 12 mmol Dabrafenib (excess)
- ~10 mL Dimethylformamide
- Purified by HPLC, lyophilized
- Quantified by HNMR, MALDI
- **Expected result** = increase in MW by 516.6 g/mol

Aim 1.1 Conjugate dabrafenib with polypeptide

Conjugating N-terminus of MMP-2 degradable peptide with carboxylic acid of DBCO

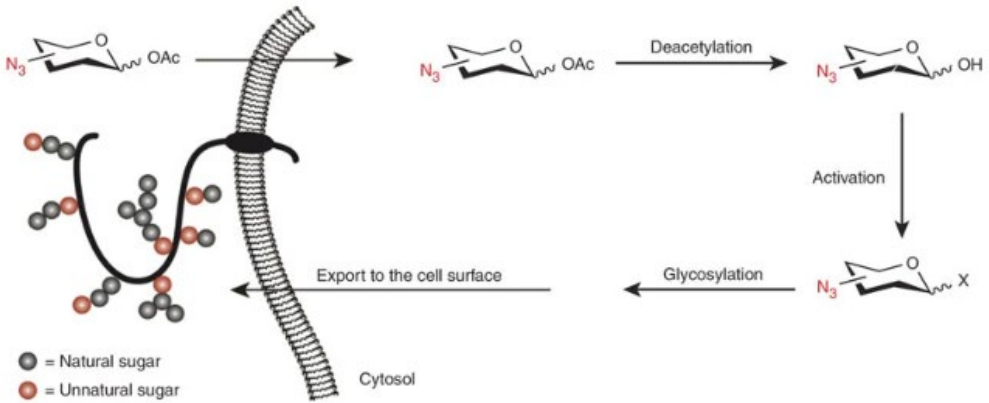


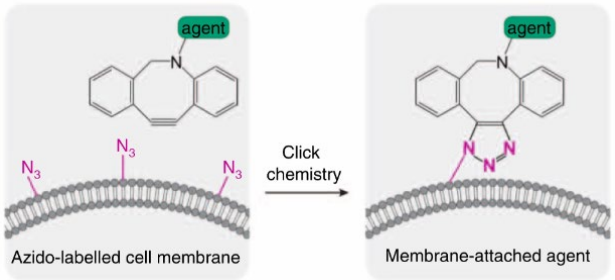
DMTMM coupling:

- 1.2 mmol MMP2 peptide
- 1.2 mmol DMTMM
- 12 mmol DBCO-PEG4-COOH
- ~10 mL Deionized water
- Purified by HPLC, lyophilized
- Quantified by HNMR, MALDI
- **Expected result** = increase in MW by 549.6 g/mol

Aim 1.2 Conjugate astrocytes with polypeptide-dabrafenib

- iPSC-derived astrocytes metabolically labeled with pendant **azides** by azido-sugar (Ac4ManAz) in culture media
  - Validated with fluorescent labeling & microscopy
- Azide-labeled astrocytes conjugated to “cellular backpacks” by **DBCO**-peptide-dabrafenib in culture media
  - **DBCO** reacts with **azides** via strain-promoted azide-alkyne cycloaddition





Wang, H. *et al.* Metabolic labeling and targeted modulation of dendritic cells. *Nat Mater* **19**, 1244-1252 (2020)

Aim 1 Engineer patient-derived astrocytes as dabrafenib-carrying “cellular backpacks” - expected results

- Sub aim 1.1
  - After tethering dabrafenib to peptide-DBCO, immobilized dabrafenib expected to have reduced bioactivity vs. unmodified dabrafenib, which will be assessed via cytotoxicity and proliferation assays using 2 MBM cell lines.
  - Comparable bioactivity expected after cleavage of carrier peptide

- Sub aim 1.2
  - After conjugating “cellular backpacks”, release rate and cumulative release expected to be more controlled/sustained vs. unmodified dabrafenib, which will be investigated using collagenase-D as a model protease in vitro.
  - Changes in astrocyte morphology, migratory speed & direction expected to be negligible, and will be investigated via real-time microscopy for before vs. after conjugation to “cellular backpacks”

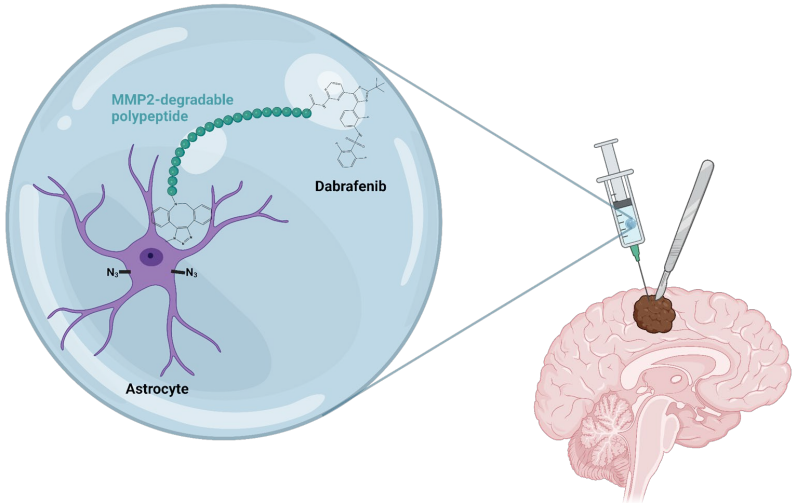
Aim 1 Potential pitfalls & alternative approaches

- Dabrafenib-containing liposomes (rather than modified dabrafenib) can be used for conjugation if post-cleavage peptide residual changes drug bioactivity
- Exploration of other BRAFi for similar bioactivity
- Upon activation by melanoma cells, astrocytes can secrete MMP-2. If non-specific self-release observed in astrocytes, CRISPR-Cas9 knockout MMP-2 expression from astrocytes

Wasilewski, D., Priego, N., Fustero-Torre, C. & Valiente, M. Reactive Astrocytes in Brain Metastasis. Front Oncol 7, 298 (2017)

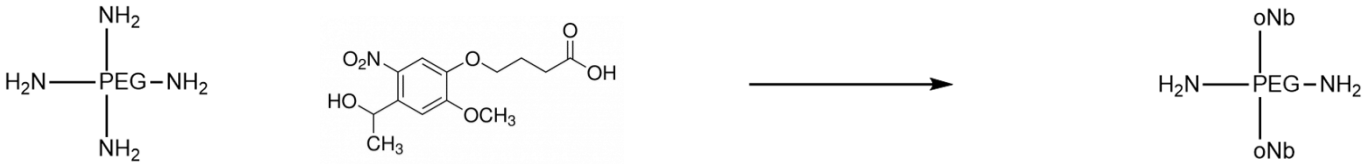
Aim 2 Encapsulate engineered astrocytes in pH-responsive microgel for in vivo delivery

- Sub aim 2.1: Encapsulate astrocyte backpack into acid-liable microgel
- Sub aim 2.2: Evaluate the efficiency of astrocyte backpack in early undetectable MBM and post-surgery tumor removal in vivo



Aim 2.1 Encapsulate astrocyte backpack into microgel

Conjugating PEG-amine with nitrobenzyl

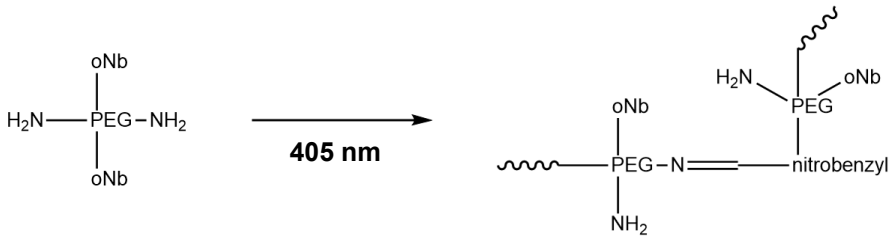


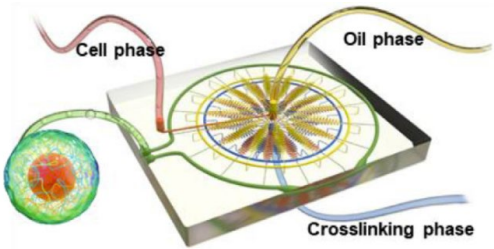
HATU coupling:

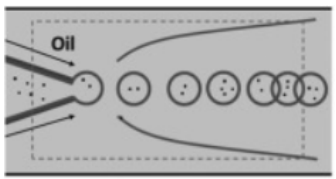
- 0.2 mmol PEG-amine
- 0.2 mmol 4-Methylmorpholine
- 3.2 mmol HATU
- 0.4 mmol Nitrobenzyl
- ~10 mL Dimethylformamide
- Purified by precip., dialysis
- Quantified by HNMR, MALDI
- **Expected result** = increase in MW by 592.5 g/mol

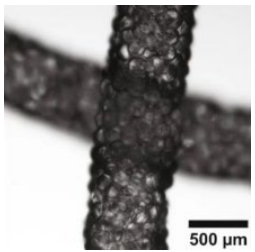
Aim 2.1 Encapsulate astrocyte backpack into microgel

Phototriggering nitrobenzyl forms ketone, subsequently forms Schiff base with free amines









- Photopolymerization & mechanical properties assessed by rheology (varying wt% polymer, light intensity, and potentially network connectivity)
- Microfluidic encapsulation of astrocytes will be investigated via efficiency, size distribution, and viability assay
- Degradation and release kinetics assessed by rheology, change in mechanics after incubation in a variety of acidic pH buffers
- Protection of astrocytes from shear-force will be assessed via viability assay

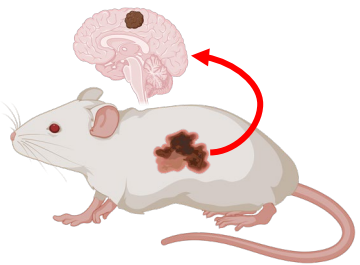
An, C. *et al.* Microfluidic-templated cell-laden microgels fabricated using phototriggered imine-crosslinking as injectable and adaptable granular gels for bone regeneration. *Acta Biomater* **157**, 91-107 (2023)

Aim 2.2 In vivo efficacy evaluation - MBM model

Dct::TVA;Braf^{V600E};
Cdkn2a$^{lox/lox}$;
Pten$^{lox/lox}$ + RCAS-Cre ×
RCAS-myrAKT1

Melanoma but
No MBM yet

79% MBM
in 6 weeks



MBM will be verified
by PET & CT

Cho, J. H. et al. AKT1 Activation Promotes Development of Melanoma Metastases. Cell Rep 13, 898-905 (2015).

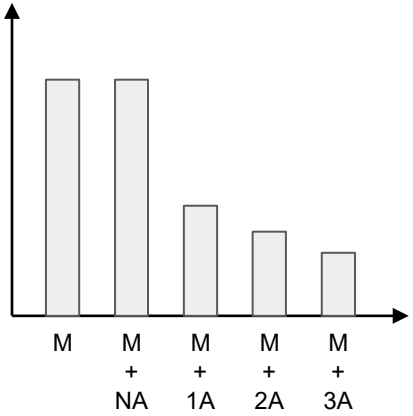
Aim 2.2 In vivo efficacy evaluation - early MBM prevention

Melanoma but no MBM yet

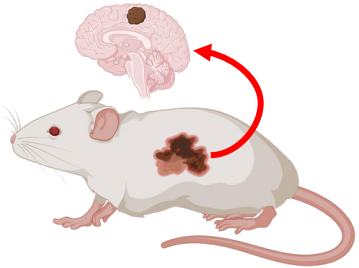
Intracranial injection

Microgel
Microgel + native astrocytes
Microgel + astrocyte backpack (1.0 ×)
Microgel + astrocyte backpack (2.0 ×)
Microgel + astrocyte backpack (3.3 ×)

MBM incidence rate



Aim 2.2 In vivo efficacy evaluation - MBM relapse prevention after surgery

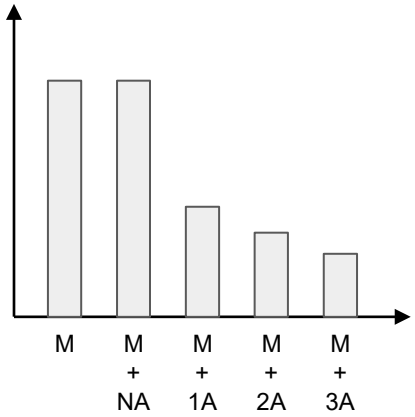


MBM verified by PET & CT

Surgical removal & intracranial injection into cavity

Microgel
Microgel + native astrocytes
Microgel + astrocyte backpack (1.0 ×)
Microgel + astrocyte backpack (2.0 ×)
Microgel + astrocyte backpack (3.3 ×)

MBM relapse rate



Aim 2 Encapsulate engineered astrocytes in pH-responsive microgel for in vivo delivery - expected results

- Sub aim 2.1
  - Microgel encapsulation expected to protect astrocyte “cellular backpacks” during injection, and provide controlled release of astrocytes for treatment of MBM, which will be accessed by an injection and viability assay

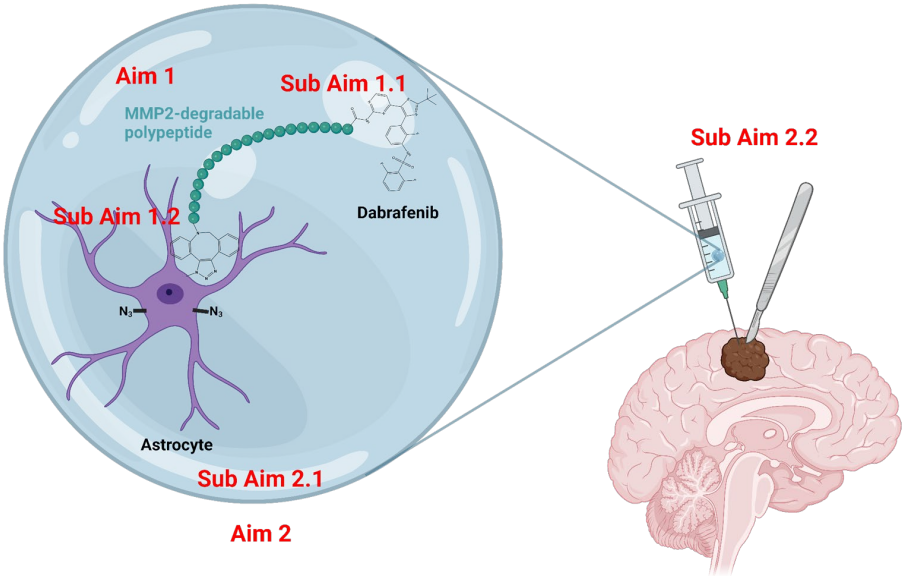
- Sub aim 2.2
  - Microgel + astrocyte backpacks expected to prevent early micro-MBM, assisted in recovery from surgical removal of macro-MBM tumor & prevent MBM relapses in in vivo assays
  - Dose-response relationship expected as astrocyte backpack concentration increases

Aim 2 potential pitfalls & alternative approaches

- Nitrobenzyl concentration varied for target functionalization (2 of 4 arms)
- T cells as alternative carrier if astrocytes loss stimulated migration capacity
- Polymer weight percentage, number of crosslinks & crosslinking mechanism can be adjusted as needed to ensure in vivo translation
- Patient-derived xenograft to generate patient-specific MBM mouse model for more effective simulation of patients with confounded background

Faria, C. C. et al. Patient-derived models of brain metastases recapitulate human disseminated disease. Cell Rep Med 3, 100623 (2022)

Summary & future directions



2-stage TME-responsive drug delivery strategy

- Optimization of dabrafenib load per astrocyte to enhance efficacy while maintain mobility
- Comparison of performance to other single-agent BRAFi & synergistic combination of BRAFi + MEKi, such as trametinib
- Investigate using 2-hydroxypropyltrimethyl ammonium chloride chitosan as microgel backbone for additional anti-tumor properties
- Further PK/PD studies before phase I clinical trial

Thank you!