Preclinical Evaluation of Radiolabelled AMG487

Hayeon (Nancy) Kim[1,2], Dominic Simpson[1], Cassis Varlow[1], Roger Raymond[1], Neil Vasdev[1], Amanda Boyle[1,2]

[1]Azrieli Centre for Neuro-Radiochemistry, Brain Health Imaging Centre, CAMH. [2]Institute of Medical Science, University of Toronto.

Introduction

AMG487 is a highly selective antagonist of C-X-C motif chemokine receptor 3 (CXCR3) that prevents its chemokine ligands CXCL10 and CXCL11 from binding to the receptor.[1] CXCR3 is a G protein-coupled receptor heavily expressed on activated immune cells that, when bound to its ligands, plays a vital role in recruiting immune cells to an injured or inflamed tissue.[2] CXCR3 expressions are involved in autoimmune diseases, transplantation, infections, and cancer.[3] CXCR3 upregulation can be imaged in vivo using its radiolabelled AMG487 with positron emission tomography (PET) to study its role in the disease model.

Objective

This study aims to preclinically assess novel radiolabelled AMG487

- *In vitro:* characterize [^{3}H]AMG487 binding properties
- *In vivo:* characterize pharmacokinetics with [^{3}H]AMG487

Methods

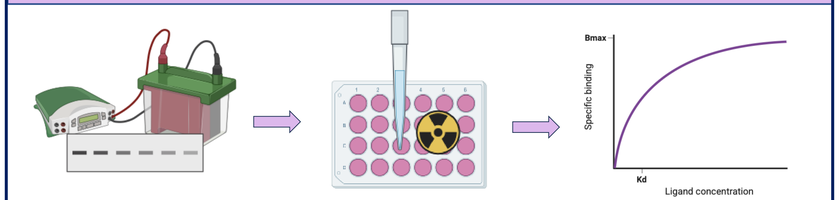


Fig 1. Experimental schematic for assessing binding properties of [^{3}H]AMG487 with Western blot and cell binding assay

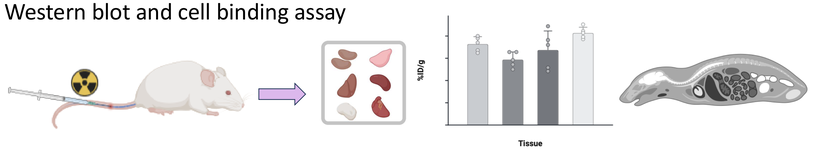


Fig 2. Experimental design outlining biodistribution and ex-vivo autoradiography to characterize pharmacokinetics with tritium labelled AMG487

Preliminary Result 1 – Western Blot

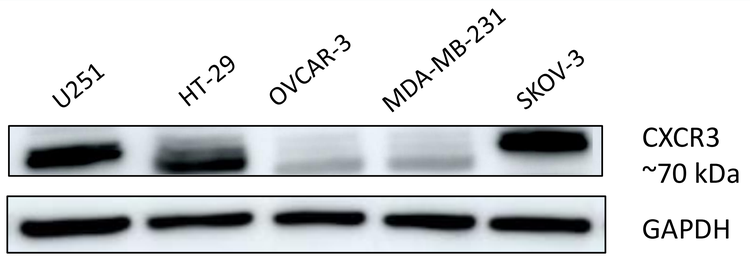


Fig 3. **CXCR3 protein expression across cancer cell lines.** Western blot showing CXCR3 expression in U251, HT-29, OVCAR-3, MDA-MB-231, and SKOV-3. GAPDH shown as loading control. (n=1)

Preliminary Result 2 – Three Concentration Binding Assay

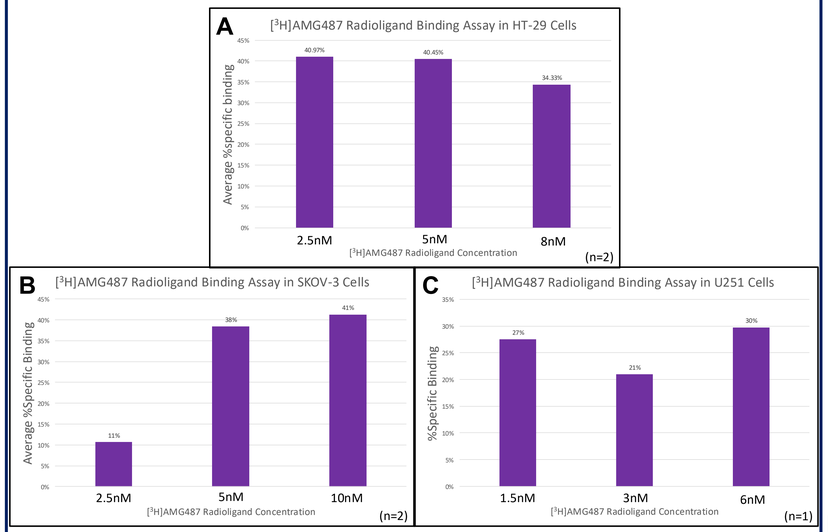


Fig 4. **Three-concentration whole cell binding assay with [^{3}H]AMG487 across HT-29, SKOV-3, and U251 cell lines.** A: HT-29 cell line with 2.5, 5, 8 nM; B: SKOV-3 cell line with 2.5, 5, 10 nM; C: U251 cell line with 1.5, 3, 6 nM of [^{3}H]AMG487. Specific binding was calculated from total binding minus nonspecific, where nonspecific binding included 1 µM of cold AMG487.

Preliminary Result 3 – [^{3}H]AMG487 Tracer Optimization

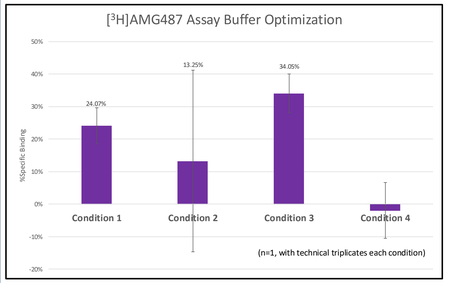
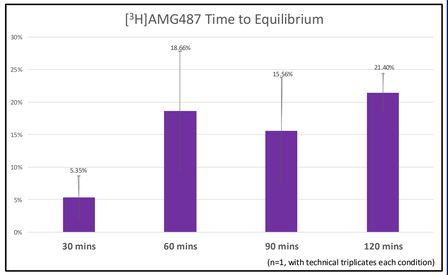


Fig 5. **Specific binding of [^{3}H]AMG487 across four buffer conditions.** Different buffers included: (1) serum-free DMEM + PBS, (2) serum-free medium + PBS + 0.1% BSA, (3) HEPES-based buffer with $CaCl_2$, $MgCl_2$, and NaCl, and (4) HEPES-based buffer + 0.1% BSA. Data represent mean ± SD of the technical triplicates.

Fig 6. **Specific binding of [^{3}H]AMG487 at increasing incubation times.** Binding was performed with HEPES-based buffer across four time points (30, 60, 90, 120 minutes). Data represent mean ± SD of the technical triplicates.



Conclusion & Future Directions

- Preliminary Western blot revealed higher CXCR3 expression in U251, HT-29, and SKOV-3 cell lines.
- One-point binding assays show low specific binding of [^{3}H]AMG487 across three cell lines, suggesting further assay optimization is required.
- Biodistribution and ex-vivo autoradiography will be conducted for in-vivo receptor binding of the radiolabelled AMG487.

References

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