Do you know where your daughters are?
ARAspheres—Biopolymer nanoparticles with peptide targeting that chelate radionuclides and retain decay daughters

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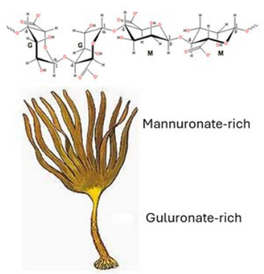
ARAspheres is a radiotherapeutic nanoparticle designed for local administration into cavitary spaces in the body such as bladder, peritoneum, and the resection cavity following surgical removal of glioblastoma. The biopolymer nanoparticles are able to chelate an alpha-emitting radionuclide and retain the decay daughters which is unique. Furthermore, the tissue diffusible nanoparticles can be conjugated with tumor-targeting peptides to seek and kill residual tumor cells

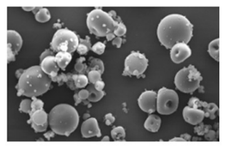
Alginate — the biopolymer

Alginate is a structural polysaccharide extracted from seaweed and kelp



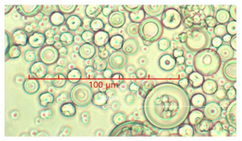
Laminaria hyperborea

Producing ARAspheres



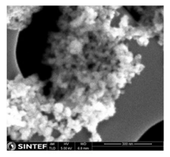
Sodium alginate (soluble) spray dried microparticles (10-12 µm)

Ca^{2+}

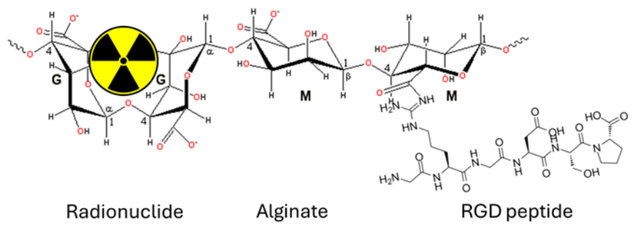


Conversion to calcium alginate (insoluble) microparticles (10-12 µm)

Bead milling to nanoparticles (60-80 nm)



ARAspheres



Radionuclide Alginate RGD peptide

ARA = actinium-RGD-alginate

Peptide targeting

Tumor type	Integrins expressed	Associated phenotype	RGD binding
Breast	$\alpha_6\beta_4/\alpha_v\beta_3$	Increase tumor size and grade	✓
Cervical	$\alpha_v\beta_3/\alpha_v\beta_6$	Decreased patient survival	✓
Colon	$\alpha_v\beta_6$	Reduced patient survival	✓
Glioblastoma	**$\alpha_v\beta_3/\alpha_v\beta_5$**	**Expressed at tumor/normal tissue margin and possible role in invasion**	✓
Melanoma	$\alpha_v\beta_3/\alpha_5\beta_1$	Promotes lymph node metastasis	✓
Non-small cell lung carcinoma	$\alpha_5\beta_1$	Decreased survival in patients with lymph node/negative tumors	✓
Ovarian	$\alpha_4\beta_1/\alpha_v\beta_3$	Increased peritoneal metastasis and tumor proliferation	✓
Pancreatic	$\alpha_v\beta_3$	Lymph node metastasis	✓
Prostate	$\alpha_v\beta_3$	Increased bone metastasis	✓

RGD peptide targets many different integrins and tumors

Other peptides specific to different cancer cell receptors can be used, such as peptides that bind to **EGFR**, **CAIX**, **FAP**, **CXCR4**, etc.

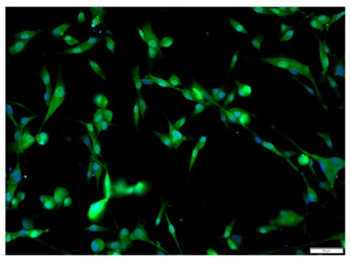
Radionuclide payload

Radionuclide	Emission	Chelation by alginate	Decay daughters bound
^{89}Sr	β	Yes	^{89}Y – stable, non-radioactive
^{177}Lu	β	Yes	^{177}Hf – stable, non-radioactive
^{223}Ra	α	Yes	^{219}Rn, ^{215}Po, ^{211}Pb, ^{211}Bi, ^{207}Tl, ^{207}Pb (stable)
^{224}Ra	α	Yes	^{220}Rn, ^{216}Po, ^{212}Pb, ^{212}Bi, ^{208}Tl, ^{208}Pb (stable)
^{227}Th	α	Yes	^{223}Ra and daughters
^{225}Ac	α	Yes	^{221}Fr, ^{217}At, ^{213}Bi, ^{213}Po, ^{209}Tl, ^{209}Pb, ^{209}Bi (stable)
Cu, Pb, Zn, Ba	stable	Yes	These non-radioactive divalent cations bind to alginate. Their radioactive isotopes will also bind, such as $^{64}Cu/^{67}Cu$

Alpha emitters (^{225}Ac, ^{223}Ra) highest energy + decay daughters add to total radiation dose

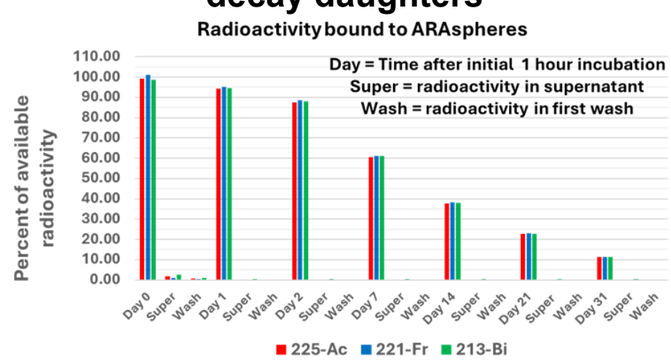
Beta emitters: longer range but lower in energy - ^{177}Lu, ^{90}Y

Proof of concept



Human glioblastoma cells (U87-MG) showing binding of fluorescent RGD-alginate (green) to cell surface integrins. Cell nuclei are stained blue

Actinium-225 binding and retention of decay daughters

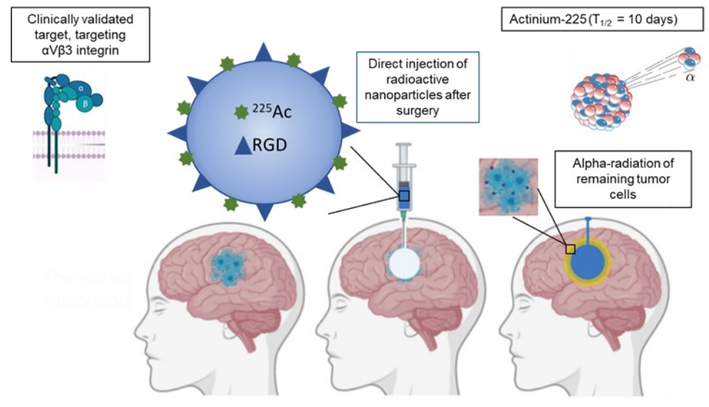


Carrier: **nanometer-sized** alginate nanoparticles which can diffuse in the brain

- The nanoparticles carry **RGD, a clinically validated targeting peptide,** which binds to specific tumor cell receptors (αvβ3 integrins), highly expressed on glioblastoma cells
- Alginate is an excellent **chelator**, retains decay daughters

Payload: alpha particle radiation emitting radionuclide (**actinium-225**), with a **$t_{1/2}$ of 10 days** and a **range** of a few cell diameters

- **Mechanism of action:** cell death by **alpha** radiation-induced unrepairable **DNA damage**



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