"Tumor targeted conjugable peptides"

Background and Description of Invention. Herein, the inventors propose to increase the tumor homing properties of albumin-based drugs and nanoparticles by an "active" targeting mechanisms, by coupling albumin with ligands selective for receptors overexpressed in the tumor vasculature such as αvβ3 heterodimer. Because of its biocompatibility, its long circulating half-life and its tendency to accumulate in tumors (owing to increased permeability and defective lymphatic drainage in neoplastic tissues) albumin is emerging as a versatile drug carrier in a number of applications in cancer therapy. Notably, albumin-paclitaxel nanoparticles (Abraxane), have been approved for the treatment of metastatic breast cancer, (www.abraxane.com) highlighting the importance of this protein as a versatile material for the successful development of new anticancer nanomedicines.

The present invention is a new head-to-tail-cyclized hexapeptide containing the isoAsp-Gly-Arg (isoDGR) motif that, after chemical conjugation to human serum albumin (HSA), recognizes αvβ3 with very good selectivity, binds to tumor vessels, inhibits tumor growth and works as an efficient ligand for the delivery of nanomedicines to tumor vasculature.

IsoDGR is a tripeptide sequence that can arise in fibronectin as a consequence of spontaneous asparagine deamidation at Asn-Gly-Arg (NGR) sites and that works as a biological switch for the regulation of cell adhesion. IsoDGR is a mimetic of Arg-Gly-Asp (RGD), involved in the regulation of cell adhesion. The inventors and other investigators have shown that isoDGR can recognize RGD dependent integrins (such as αvβ3, αvβ5, αvβ6, αvβ8 and α5β1) with different affinity and selectivity, depending on isoDGR conformation and molecular scaffold. To fulfill these aims the inventors have designed a series of head-to-tail-cyclized isoDGR penta and hexa-peptides containing a free thiol group and analyzed their integrin binding properties before and after conjugation to proteins and nanoparticles. Peptide-albumin conjugates were positively tested for (i) integrin binding properties, (ii) capability to recognize the endothelial lining of tumor vessels and (iii) anti-cancer activity in mouse fibrosarcoma and lymphoma models (*Curnis, Corti et al. IsoDGR-tagged albumin: a new αvβ3 selective carrier for nanodrug delivery to tumors. Small 2012; Curnis et al., NGR-tagged nano-gold: A new CD13-selective carrier for cytokine delivery to tumors. Nano Research 2016).*

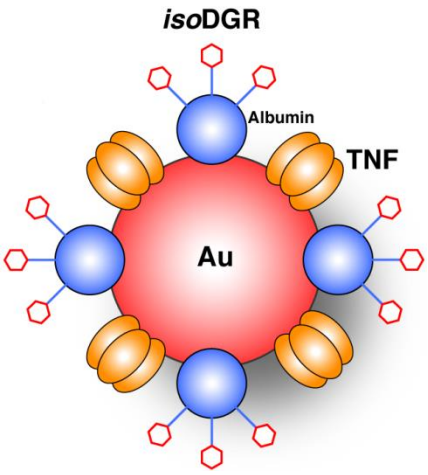

Figure 1. Gold nanoparticles (Au) loaded with isoDGR-albumin (1-HSA) and TNF.

Patent information. The international patent application was published as WO2013140317. Patent granted in US. A further European patent application, from the same Inventors, has been filed and relates to NGR modified motif (e.g. N-methyl glycine asparagine) having tumor-homing properties, preventing asparagine-deamidation without impairing CD13 recognition (Corti et al., AFM 2017). The patented technology is available for licensing worldwide.

Stage of Development. The inventors have identified a cyclic hexapeptide (called isoDGR#1) that, after coupling to human serum albumin (HSA), has a very good selectivity for αvβ3, binds to tumor vessels (**Figure 2**) and inhibits tumor growth. Furthermore, *in vivo* studies in mice bearing WEHI fibrosarcomas showed that coupling the isoDGR#1-HSA conjugate (called 1-HSA) to TNF-bearing gold nanoparticles (25 nm) (**Figure 1**), a known tumor vessel damaging agent, enhanced the anti-tumor activity of this nanomedicine more efficiently than coupling with HSA. Notably, doses of this nanomedicine equivalent to 5 pg of bioactive TNF was sufficient to induce significantly delay of tumor growth whereas "non-targeted" TNF was inactive (**Figure 3**).

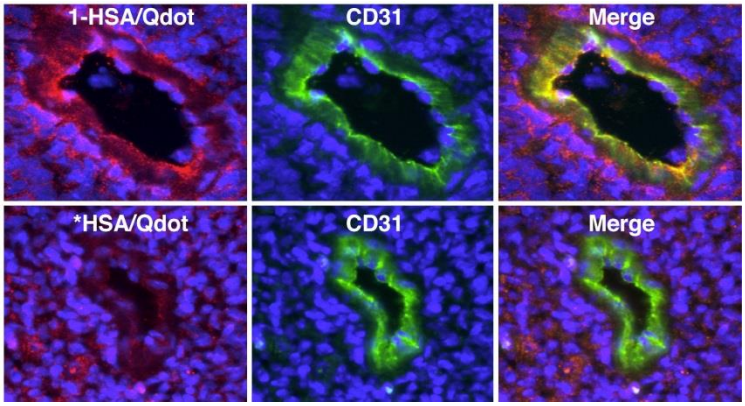

Figure 2. 1-HSA/Qdot, but not HSA/Qdot, binds to endothelial lining of tumor vessels on murine RMA lymphoma tissue sections. Frozen sections were incubated with 1-HSA, or control HSA, chemically coupled to fluorescent quantum dot nanoparticles (1-HSA/Qdot and ∗HSA/Qdot), and immunostained with anti-CD31 antibody (a marker of endothelial cells). *Red*, Qdots; *blue*, DAPI; *green*, CD31.

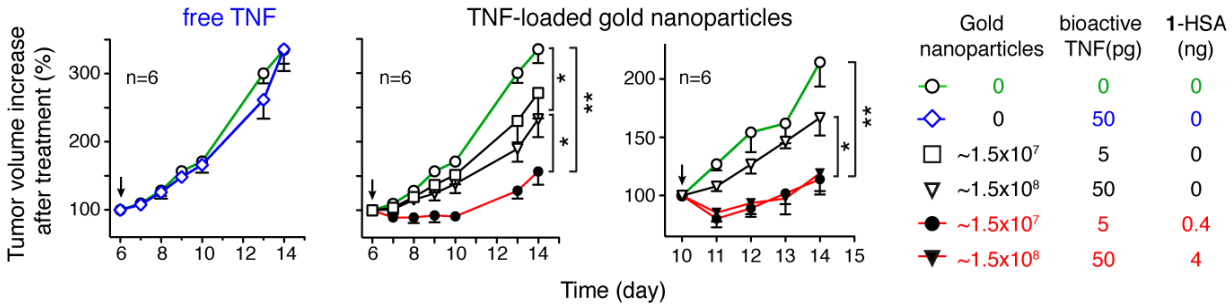

Figure 3. Coupling 1-HSA to TNF-loaded gold nanoparticles could enhance their anti-tumor activity. Significantly lower effects were observed with an equivalent dose of gold nanoparticle bearing TNF alone, or even with 10-fold higher doses. Equivalent doses of free TNF were completely inactive.

Potential Applications and Competitive Advantages. Because of its good selectivity for tumor vessels and its inherent anticancer activity the 1-HSA conjugate might be exploited as a novel and versatile material for the preparation of a wide range of tumor vasculature-selective drugs and nanoparticles for cancer therapy and diagnosis.

Higher selectivity. Considering that αvβ8 is expressed in yolk sac, placenta, brain perivascular astrocytes, Schwann cells, renal glomerular mesangial cells and pulmonary epithelial cells and that αvβ6 is expressed in epithelia, the higher selectivity of 1-HSA for integrins expressed in tumor vessels might represent an important advantage. Interestingly, both linker and protein scaffold markedly contribute to the selective recognition of αvβ3 by 1-HSA. Enhancement of binding affinity and selectivity was observed also after coupling peptide isoDGR #1 to avidin.

No toxicity. 1-HSA did not cause loss of body weight or evident toxic reactions at any tested dose.

These results, overall, suggest that isoDGR-tagged albumin is a new vascular targeting agent that might be exploited in place of albumin for the preparation of new nanotherapeutics and nanodiagnostics with improved tumor homing ability.

Of note, even the peptide isoDGR#1 (uncoupled, with a free thiol group) can be exploited in principle as a ligand for the functionalization of a number of therapeutic and diagnostic compounds and nanoparticles thereby improving their tumor homing ability (*Corti, Curnis, et al. Peptide-mediated targeting of cytokines to tumor vasculature: the NGR-hTNF example. BioDrugs. 2013*)

We seek a potential commercial partner focused on tumor-targeted therapies for enhancing efficacy of anti-tumor drugs.

For further information on this project please contact:

Business Contact
Paola Vella
Head, Office of Biotechnology Transfer
San Raffaele Hospital and Scientific Institute
Tel: +39 02 2643 4281
Fax: + 39 02 2643 8138
E-mail: vella.paola@hsr.it

Scientific Contact
Prof. Angelo Corti
Tumor Biology and Vascular Targeting Unit
San Raffaele Hospital and Scientific Institute
Tel: +39 02 2643 4802
Fax: + 39 02 2643 4786
E-mail: corti.angelo@hsr.it