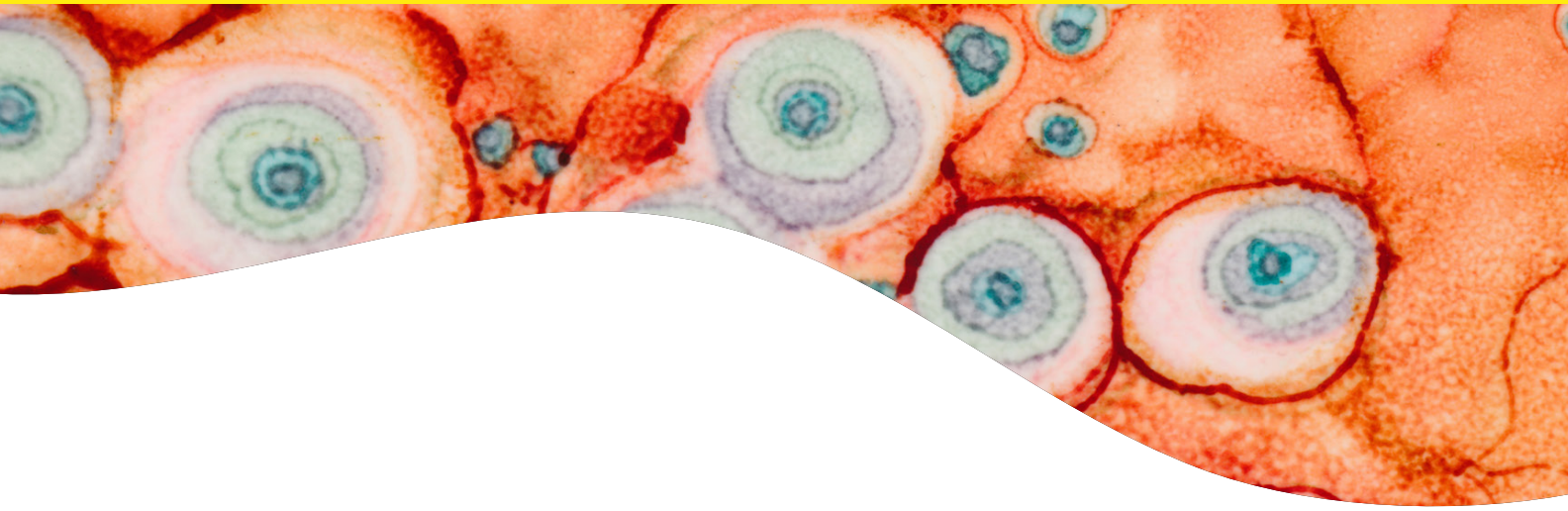
Integrins – A promising target for new treatment approaches in atherosclerosis and cancer.



As our understanding of the role of integrins in diseases such as atherosclerosis and cancer deepens, the rationale for researching integrins as a druggable target becomes more apparent. Developing integrin-blocking drugs and exploring their potential could facilitate new treatment options for diseases where the over-expression of integrin contributes to thrombotic complications or facilitates angiogenesis. Further investigation may prove especially worthwhile in immuno-oncology, where integrins are part of the mechanism that enables cancer to evade the immune system. Enabling the body's own innate immune response to overcome these immune-cell-evading mechanisms, and detect and kill cancer cells by blocking integrins could advance the field of immuno-oncology.

What are integrins?

Integrins are cell surface signaling molecules that are essential for regulating communication between cells and their microenvironment. Critically involved in the cells' ability to adhere to the extracellular matrix as well as in cellular migration, they are important to maintaining the integrity of healthy tissue. Abnormal expression of integrins occurs in diseases such as atherosclerosis, where they regulate atherosclerotic plaque development, and cancer, where integrin expression correlate with tumor aggressiveness. In particular, αvβ3 integrin is significantly upregulated in tumor cells and activated endothelial cells during neoangiogenesis. It is also a critical regulator of PD-L1, a protein that plays a major role in helping tumor cells evade the immune system, where depletion of αvβ3 integrin has been shown to impair tumor growth as well as elicit immunotherapeutic protection.[1] Therefore, the development of novel anti-αvβ3 integrin therapies to block their activity *in vivo* is a valid therapeutic pursuit.

To develop such therapies, the ability to spatially and temporally visualize as well as strictly quantify changes in the expression of αvβ3 integrins as distinct biomarkers of tumor development and metastasis would be highly useful. Radionuclide-labeled molecules to bind integrins have been developed for positron emission (PET) and Single-Photon Emission Computed Tomography Imaging (SPECT). These agents bond the Arginine, Glycine, and Aspartate (RGD) site on integrins where

they adhere to the extracellular matrix, but off target binding of RGD peptide-based agents can confound the ability to strictly quantify αvβ3 integrin levels *in vivo*. In addition, while these long-established and non-invasive imaging modalities are sensitive and provide functional information, they are limited by their use of ionizing radiation with its associated costs, complexity, short half-lives, and radioactive material handling/disposal.

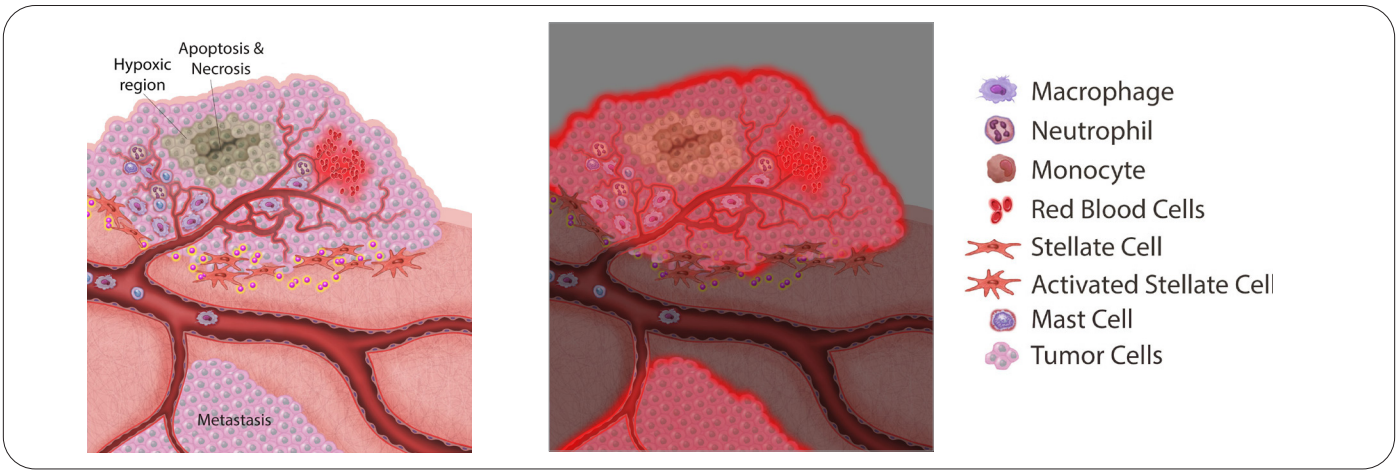


Figure 1. αvβ3 integrin is significantly upregulated in tumor cells and correlates with tumor aggressiveness. IVISense Integrin Receptor fluorescent imaging of fine tumor definition is represented in the figure on the right.

Alternatively, optical imaging as a non-ionizing imaging method offers multiple advantages but has also been limited by a lack of a high affinity αvβ3 integrin probe. Similar to PET and SPECT, RGD peptide-based agents have been used as optical imaging probes. However they fail to precisely image and quantify signal of αvβ3 receptor binding, instead detecting and binding to multiple other confounding integrins.

In contrast, the use of a high affinity and selective probe, such as IVISense™ Integrin Receptor fluorescent probe over RDG peptide-based agents, provides improved circulation half-life and specificity which allows for precise detection of αvβ3 integrins. As a distinct biomarker of atherosclerosis and cancer progression, αvβ3 integrin imaged with IntegriSense can provide excellent tumor definition with minimal off-target distribution. As a druggable target for immunotherapy, αvβ3 integrin and its role in immune system evasion can be non-invasively investigated *in vivo* within the context of the tumor microenvironment using IVISense Integrin Receptor.

What is IVISense Integrin Receptor Probe?

IVISense Integrin Receptor is a NIR fluorescent imaging probe used for *in vivo* detection of αvβ3 integrin. This validated, high affinity (Kd = 4.2 nM) fluorescent probe, with selectivity 5-20x over RDG peptide-based agents for αvβ3 integrin, can provide precise detection of αvβ3 integrins. IVISense Integrin Receptor probe is a low molecular weight peptidomimetic antagonist coupled to a red fluorochrome to selectively target αvβ3 integrin for non-invasive imaging of disease status and progression. It enables *in vivo* and *in vitro* visualization and quantification of integrin expression in tumor cells as well as in neovasculature. IVISense Integrin Receptor probe is highly effective in monitoring tumor growth and tumor angiogenesis, and for assessing treatment efficacy, where IntegriSense can be used to determine the coverage of an integrin-blocking treatment.

How is IVISense Integrin Receptor probe used?

Example publications in Immuno-oncology/Immunotherapy Research Using IVISense Integrin Receptor fluorescent probes:

- Garetto S, Sardi C, Martini E, et al. Tailored chemokine receptor modification improves homing of adoptive therapy T cells in a spontaneous tumor model. italicize publication. 2016;7(28):43010-43026. doi:10.18632/oncotarget.9280. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5190004/
- Su X, Esser AK, Amend SR, et al. Antagonizing Integrin β3 Increases Immunosuppression in Cancer. italicicze.2016;76(12):3484-3495. doi:10.1158/0008-5472. CAN-15-2663. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4944657/

Case Studies demonstrating additional applications using IVISense Integrin Receptor fluorescent probes:

Case study 1

Response to anti-angiogenic cancer therapy compound tracked by fluorescence tomography

Tumors were established by injecting human-derived A673 neuroepithelioma cells into the flanks of immunodeficient mice. Mice received two treatments of avastin (an anti-angiogenic compound that inhibits the binding of VEGF to its cell surface receptors) or vehicle per week. On day seven, mice were administered IVISense Integrin Receptor and imaged 24 hours later. As αvβ3 integrins are highly expressed during the formation of new vasculature, the fluorescent images indicate that the animals in the avastin treatment group had decreased neovascularization, as shown by decreased fluorescent signal due to less IVISense Integrin Receptor binding (Figure 2A). When compared against standard caliper measurements, IVISense Integrin Receptor imaging and quantification provided consistent and comparable results (Figures 2B and 2C).

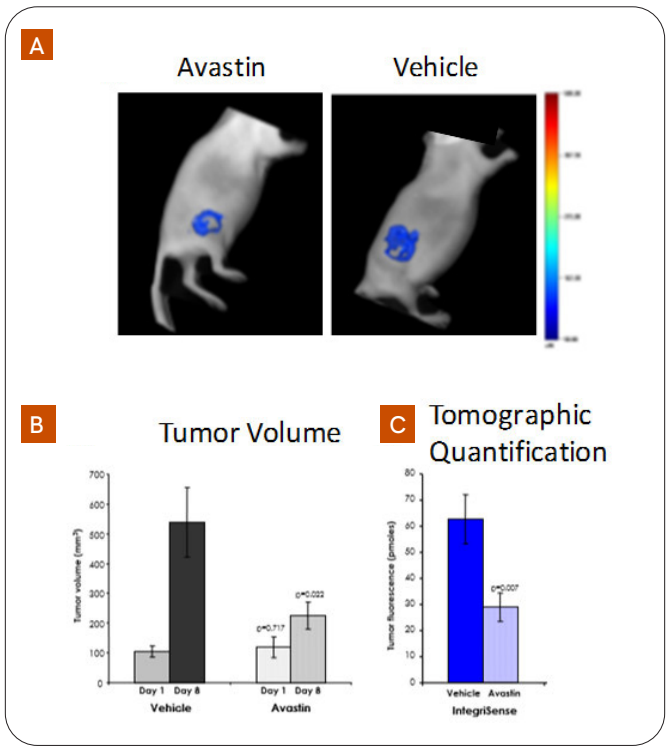


Figure 2. Kossodo et al. "Dual ***In Vivo*** Quantification of Integrin-targeted and Protease-activated Agents in Cancer Using Fluorescence Molecular Tomography (FMT®)", ***Mol Imaging Biol*** (2009).

Case study 2

Atherosclerosis and vascular inflammation

Inflammation is a major contributor to pathological effects in the development of atherosclerotic lesions. Atherosclerosis-prone apolipoprotein E-deficient mice have poor lipoprotein clearance and accumulate high levels of cholesterol in the blood resulting in vascular inflammation and atherosclerotic plaque formation. When fed a high fat diet, these mice begin to exhibit atherosclerotic lesions in as few as 15 weeks. αvβ3 integrins are expressed at the sites of inflammation and atherosclerotic lesions. In Figure 3(1A), *in vivo* imaging shows integrin expression and atherosclerosis as detected by IVISense Integrin Receptor probe in the aortic root, arch, and carotid arteries. Figure 3(1B) shows damage to the proximal part of the descending aorta. In Figure 3(2A), *ex vivo* images of dissected arteries confirm observations of *in vivo* images. The correlation between *in vivo* and *ex vivo* measurements are shown in Figure 3(2B).

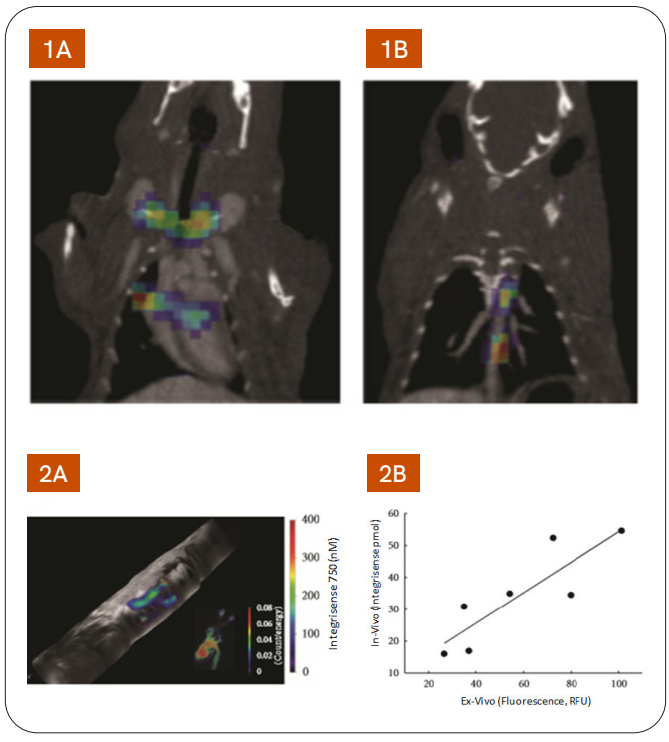


Figure 3. Lin et al., Quantitative Longitudinal Imaging of Vascular Inflammation and Treatment by Ezetimibe in apoE Mice by FMT Using New Optical Imaging Biomarkers of Cathepsin Activity and αvβ3 Integrin. Int. J. of Mol. Imaging Volume 2012, Article ID 189254.

Use IVISense Integrin Receptor Probe as part of a complete experimental solution package

Revvity provides complete *in vivo* imaging solutions including reagents, instrumentation, and support expertise to help you better understand the progression of diseases and their related processes, or to evaluate the potential therapeutic efficacy of drugs targeting the underlying mechanisms involved in disease.

IVISense Integrin Receptor fluorescent probes are available in three wavelengths; 645, 680, and 750, and are designed to target αvβ3 integrin *in vitro* and *in vivo*.

Cat #	Product	
NEV10640	IVISense Integrin Receptor 645	Target αvβ3 integrin expressed in oncology, atherosclerosis, and angiogenesis disease models.
NEV10645	IVISense Integrin Receptor 680	
NEV10873	IVISense Integrin Receptor 750	

Reference

1. αvβ3-integrin regulates PD-L1 expression and is involved in cancer immune evasion.Andrea Vannini, Valerio Leoni, Catia Barboni, Mara Sanapo, Anna Zaghini, Paolo Malatesta, Gabriella Campadelli-Fiume, Tatiana Gianni.Proceedings of the National Academy of Sciences Oct 2019, 116 (40) 20141-20150; DOI: 10.1073/pnas.1901931116.

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