# Visualizing gelsolin amyloid with nanobodies; small molecules with big potential

Amyloidosis is a group of diseases characterized by the deposition of aggregated proteins, or protein fragments, in tissues and organs. The diseases differ from each other in the causative protein, but once aggregation starts, they all result in the deposition of amyloid plaques. Gelsolin amyloidosis or AGel is one of these diseases and is caused by a mutation in a gene coding for a protein known as gelsolin. Due to this, this protein can no longer adopt its natural structure, resulting in a breakdown process by various proteases. Proteases are proteins capable of degrading other proteins and are indispensable for normal physiology. In the case of gelsolin however, the protease activity results in a tiny fragment capable of aggregating. Over time, deposits of these gelsolin fragments form dense plaques in patients' tissues. AGel patients experience a triad of ophthalmological, neurological and dermatological symptoms. At the moment no cure is available.

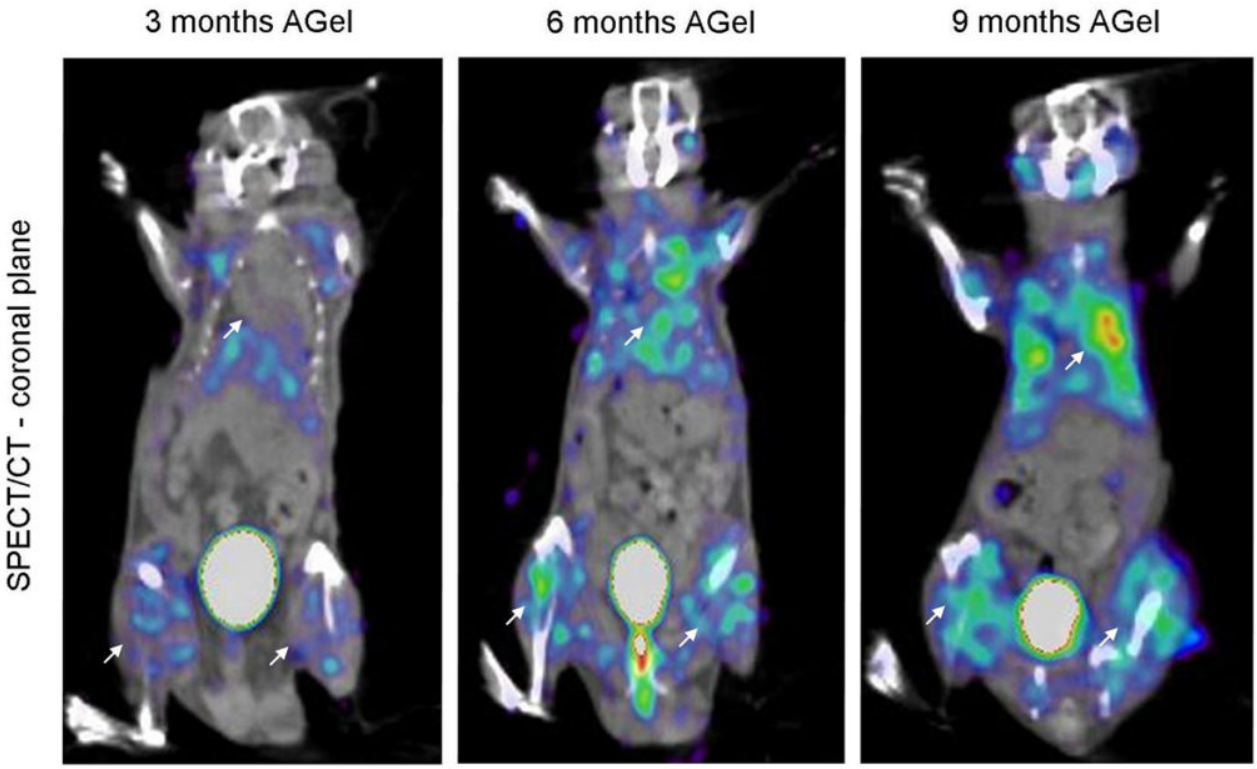


Fig. 1. Beacon FAF Nb1 shows quantitative features. In vivo medical imaging with radioactive FAF Nb1, in AGel mice, revealed its quantitative features. With age, the gelsolin amyloid buildup increases in FAF mice. The signal intensities from the FAF Nb1 beacon clearly depict this progression, as can be seen by comparing images taken in 3 month (left), 6 month (middle) and 9 month (right) old AGel mice. This is especially notable in the heart and hind leg muscles (white

arrows). Signal intensities are depicted from low to high in a color scale (blue to red).

We have developed molecular beacons (nanobodies) to monitor, in vivo, the response of AGel mice to therapeutics currently under development. Up until now, laboratory animals needed to be euthanized in order to assess the therapeutic efficacy of a drug. AGel mice contain the human mutated gelsolin gene and experience similar gelsolin plaque buildup, as observed human patients. Nanobodies are small fragments of a special type of antibodies first discovered in Camels.

In a previous study, three of these molecular beacons, capable of binding to the pathogenic gelsolin fragment, were generated (FAF Nb1, 2 and 3). Here we coupled them to a radioactive moiety ($^{99m}$Tc-isotope). After injection in AGel mice, these radioactive beacons recognize and bind to the gelsolin amyloidogenic buildup. The coupled radioactive moiety emits gamma radiation which can be visualized, in 3D, using medical imaging techniques (SPECT/CT). Hence all organs and tissues affected with gelsolin amyloid can be identified. Especially the beacon ‘FAF Nb1’ was capable of clearly visualizing gelsolin amyloid in the heart and skeletal muscle, without any significant background signal.

Data acquisition in 3, 6 and 9 month old AGel mice revealed that, besides rendering good quality images, the molecular beacons also possessed quantitative features (Fig. 1). Both in the heart and skeletal muscle, the signals intensified with age in a similar fashion as the gelsolin amyloid buildup increased (Fig. 1). Beacon ‘FAF Nb1’ showed the highest response towards gelsolin amyloid increase.

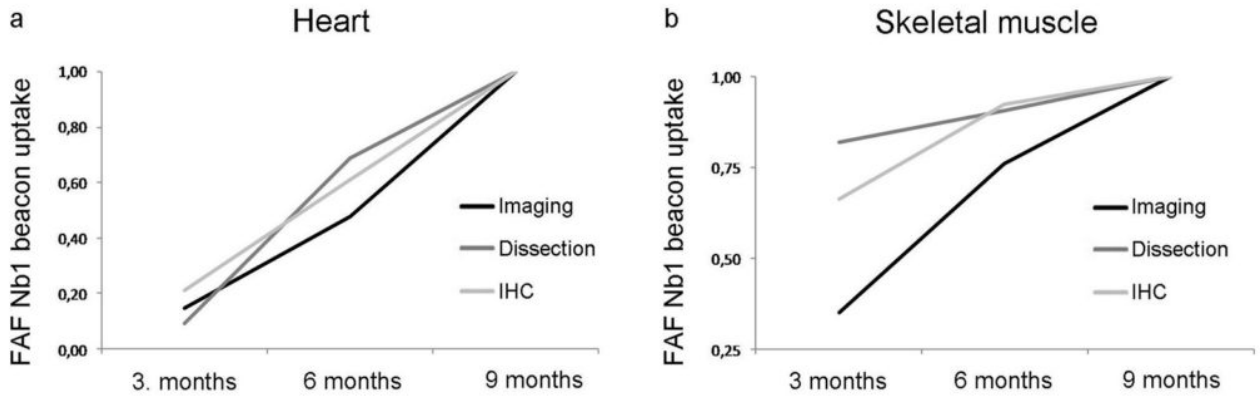


Fig. 2. Comparison of AGel amyloid buildup quantification techniques. The direct quantification of medical images obtained with the FAF Nb1 beacon was compared to the more standard immunohistochemistry and dissection analysis. In the heart (a) our imaging beacon performs equally as well. In skeletal muscle (b) FAF Nb1 imaging show a wider dynamic range compared to dissection and immunohistochemistry.

As proof of concept, medical imaging with beacon ‘FAF Nb1’ was applied in a therapeutic intervention study. In this study the therapeuticum was a molecular chaperone know to bind to mutant gelsolin, thereby hindering the proteases. Consequently less pathogenic gelsolin fragments are formed, resulting, more downstream, in a lower gelsolin amyloid buildup. Between 3 and 11 months of age the mice underwent a bimonthly medical scan. At 7 and 11 months, a subgroup was sacrificed. Their heart and skeletal muscle tissue was fluorescently stained for gelsolin amyloid. Quantification of both the medical images and fluorescently stained samples revealed a similar increase in gelsolin amyloid burden, thereby confirming that imaging with beacon ‘FAF Nb1’ can be applied in a quantitative manner during therapeutic development (Fig. 2). This new, non invasive way of screening the efficacy of potential therapeutics may speed up their development process. Moreover, research suggests that these molecular beacons are easily tolerated by humans and may therefore also prove valuable in the clinic to monitor gelsolin amyloid burden in patients.

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## Publication

Non-Invasive Imaging of Amyloid Deposits in a Mouse Model of AGel Using 99mTc-Modified Nanobodies and SPECT/CT.
Verhelle A, Van Overbeke W, Peleman C, De Smet R, Zwaenepoel O, Lahoutte T, Van Dorpe J, Devoogdt N, Gettemans J
*Mol Imaging Biol. 2016 Apr 29*