

Enhancing PET Signal at Target Tissue in Vivo: Dendritic and Multimeric Tris(hydroxypyridinone) Conjugates for Molecular Imaging of $\alpha_v\beta_3$ Integrin Expression with Gallium-68

Supplementary material

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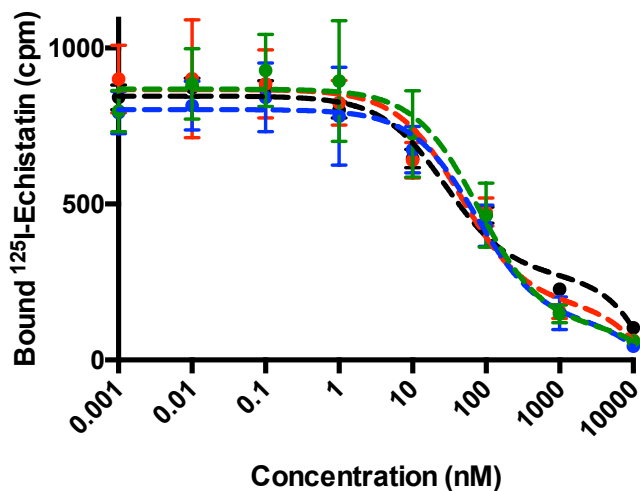


Figure S1. Mean concentration response curve for ^{125}I -echistatin titrated with c(RGDfK) (black), $[\text{Ga}(\text{HP}_3\text{-RGD})]$ (red), $[\text{Ga}(\text{HP}_3\text{-RGD}_3)]$ (blue) and $[\text{Ga}_3(\text{HP}_9\text{-RGD}_3)]$ (green). Error bars correspond to standard error of the mean; $n = 6$ for each concentration.

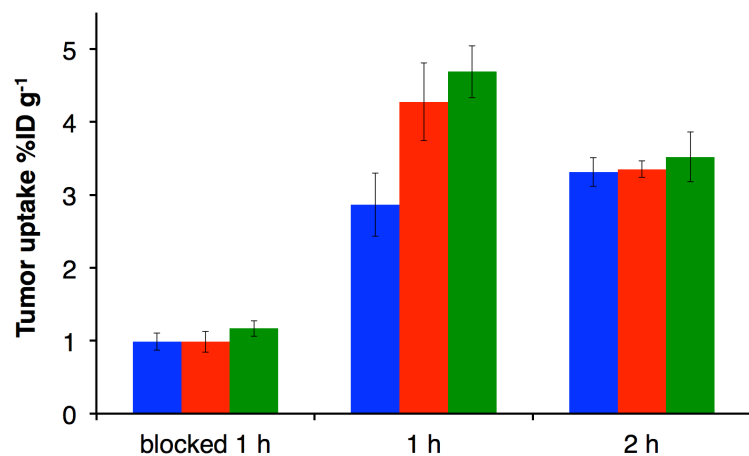


Figure S2. Comparative tumor uptake in mice bearing $\alpha_v\beta_3$ integrin-positive U87MG tumors administered ^{68}Ga -labeled conjugates 1 h PI of coadministered c(RGDfK) (blocked), and 1 and 2 h PI: $[\text{Ga}(\text{HP}_3\text{-RGD})]$ (blue), $[\text{Ga}(\text{HP}_3\text{-RGD}_3)]$ (red), $[\text{Ga}(\text{HP}_9\text{-RGD}_3)]$ (green). Error bars correspond to standard error of the mean; $n = 3$.

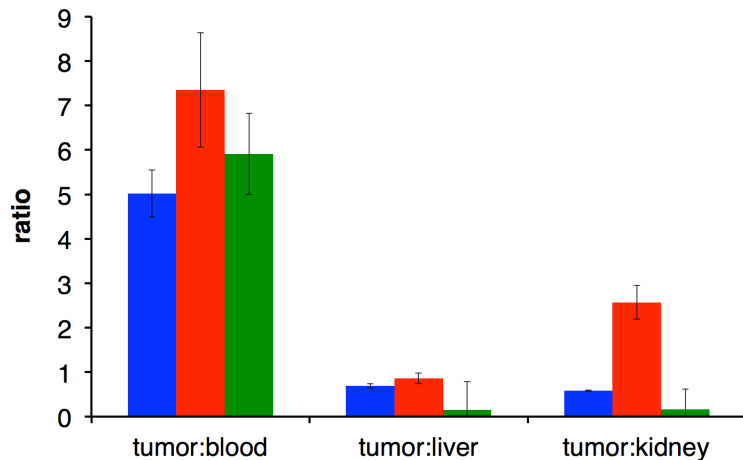


Figure S3. Tumor to blood or non-target organ ex vivo biodistribution ratios in mice bearing $\alpha_v\beta_3$ integrin-positive U87MG tumors administered ^{68}Ga -labeled conjugates 1 h PI of $[\text{Ga}(\text{HP}_3\text{-RGD})]$ (blue), $[\text{Ga}(\text{HP}_3\text{-RGD}_3)]$ (red), $[\text{Ga}(\text{HP}_9\text{-RGD}_3)]$ (green). Error bars correspond to standard error of the mean; $n = 3$.

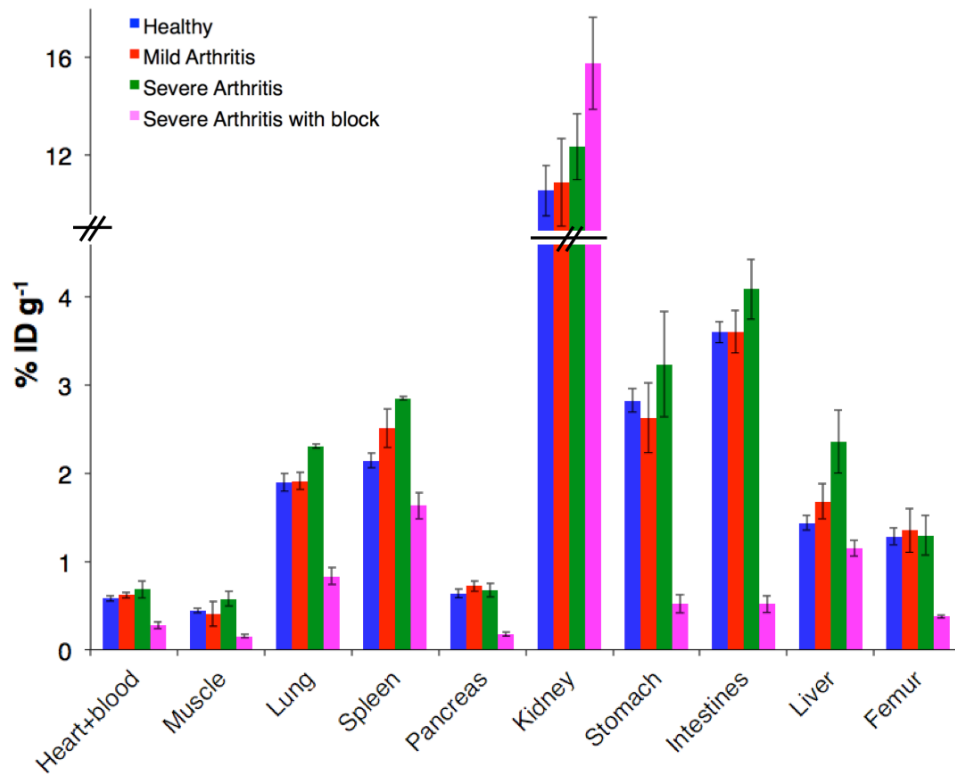


Figure S4. Ex vivo biodistribution of healthy mice (blue), mice with induced mild rheumatoid arthritis (red) and mice with induced severe rheumatoid arthritis (green), administered [$^{68}\text{Ga}(\text{HP}_3\text{-RGD}_3)$] (8 - 13 MBq). A blockade study was also undertaken in mice with induced severe rheumatoid arthritis, in which [$^{68}\text{Ga}(\text{HP}_3\text{-RGD}_3)$] was co-administered with c(RGDfK) (pink). Error bars correspond to standard error of the mean; $n = 3$.

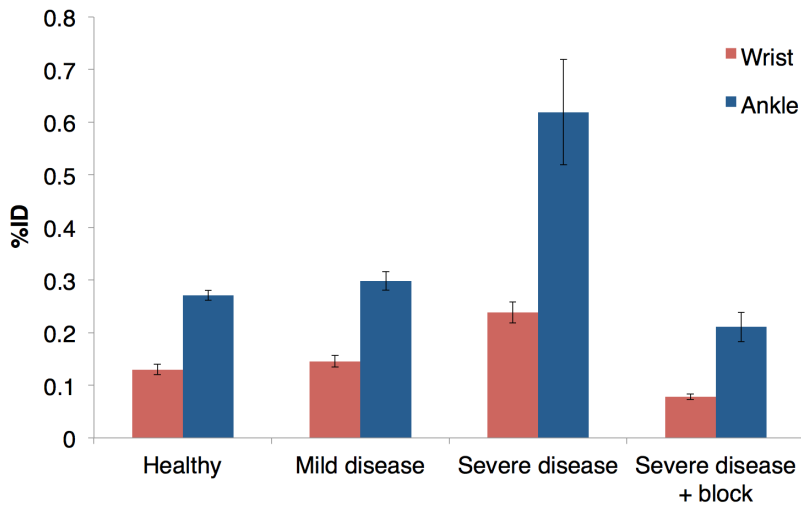


Figure S5. Ex vivo biodistribution of wrist joints (red) and ankle joints (blue) of mice administered [$^{68}\text{Ga}(\text{HP}_3\text{-RGD}_3)$] (8 - 13 MBq). Mice are grouped according to the severity of induced disease. Error bars correspond to standard error of the mean; $n = 6$ (3 animals per group, each with two ankles and two wrists).

Table S1 (a). Ex vivo biodistribution of mice bearing $\alpha_v\beta_3$ integrin-positive U87MG tumors administered [$^{68}\text{Ga}(\text{HP}_3\text{-RGD})$], $n = 3$.

Organ/tissue	1 h PI, %ID g ⁻¹ ($\pm$ SD)	With block, 1 h PI, %ID g ⁻¹ ($\pm$ SD)	2 h PI, %ID g ⁻¹ ($\pm$ SD)
Blood	0.57 $\pm$ 0.04	1.29 $\pm$ 0.15	0.25 $\pm$ 0.03
Tumor	2.86 $\pm$ 0.61	0.99 $\pm$ 0.17	3.32 $\pm$ 0.28
Muscle	0.56 $\pm$ 0.08	0.38 $\pm$ 0.06	0.36 $\pm$ 0.01
Liver	4.09 $\pm$ 0.47	2.68 $\pm$ 0.17	4.96 $\pm$ 0.33
Kidney	4.91 $\pm$ 1.05	7.39 $\pm$ 2.61	4.56 $\pm$ 0.63
Spleen	1.73 $\pm$ 0.29	0.82 $\pm$ 0.05	2.07 $\pm$ 0.07
Heart	0.92 $\pm$ 0.16	0.72 $\pm$ 0.03	0.66 $\pm$ 0.13
Lungs	1.77 $\pm$ 0.22	1.38 $\pm$ 0.34	1.59 $\pm$ 0.20

Table S1 (b). Ex vivo biodistribution of mice bearing $\alpha_v\beta_3$ integrin-positive U87MG tumors administered [$^{68}\text{Ga}(\text{HP}_3\text{-RGD}_3)$], $n = 3$.

Organ/tissue	1 h PI, %ID g ⁻¹ ($\pm$ SD)	With block, 1 h PI, %ID g ⁻¹ ($\pm$ SD)	2 h PI, %ID g ⁻¹ ($\pm$ SD)
Blood	0.67 $\pm$ 0.05	0.73 $\pm$ 0.20	0.22 $\pm$ 0.05
Tumor	4.28 $\pm$ 0.75	0.98 $\pm$ 0.20	3.35 $\pm$ 0.16
Muscle	0.78 $\pm$ 0.09	0.21 $\pm$ 0.06	0.47 $\pm$ 0.08
Liver	5.69 $\pm$ 0.10	4.36 $\pm$ 0.26	4.50 $\pm$ 0.50
Kidney	12.29 $\pm$ 0.39	15.64 $\pm$ 3.44	9.69 $\pm$ 1.61
Spleen	3.14 $\pm$ 0.62	1.17 $\pm$ 0.44	1.88 $\pm$ 0.89
Heart	1.67 $\pm$ 0.38	0.47 $\pm$ 0.16	0.89 $\pm$ 0.28
Lungs	3.24 $\pm$ 1.19	0.97 $\pm$ 0.61	2.21 $\pm$ 0.12

Table S1 (c). Ex vivo biodistribution of mice bearing $\alpha_v\beta_3$ integrin-positive U87MG tumors administered [$^{68}\text{Ga}(\text{HP}_9\text{-RGD}_3)$], $n = 3$.

Organ/tissue	1 h PI, %ID g ⁻¹ ($\pm$ SD)	With block, 1 h PI, %ID g ⁻¹ ($\pm$ SD)	2 h PI, %ID g ⁻¹ ($\pm$ SD)
Blood	0.80 $\pm$ 0.10	1.40 $\pm$ 0.22	0.67 $\pm$ 0.14
Tumor	4.69 $\pm$ 0.50	1.17 $\pm$ 0.16	3.52 $\pm$ 0.48
Muscle	1.09 $\pm$ 0.14	0.49 $\pm$ 0.07*	0.60 $\pm$ 0.03
Liver	32.37 $\pm$ 4.18	33.81 $\pm$ 1.87	35.02 $\pm$ 1.64
Kidney	28.32 $\pm$ 5.32	57.37 $\pm$ 9.39	19.98 $\pm$ 1.38
Spleen	11.40 $\pm$ 1.65	8.74 $\pm$ 1.67	8.28 $\pm$ 0.36
Heart	1.65 $\pm$ 0.25	0.94 $\pm$ 0.19	1.07 $\pm$ 0.02
Lungs	12.73 $\pm$ 2.00	2.85 $\pm$ 0.73	8.42 $\pm$ 1.98

* $n = 2$

Table S2. Ex vivo biodistribution of mice with induced severe rheumatoid arthritis administered [$^{68}\text{Ga}(\text{HP}_3\text{-RGD}_3)$] without and with co-administration of cRGDFK, $n = 3$.

Organ/tissue	1 h PI, %ID g^{-1} ($\pm$ SEM)	With block, 1 h PI, %ID g^{-1} ($\pm$ SEM)	Mean difference (95% CI), %ID g^{-1}	<i>p</i> value
Blood	0.68 $\pm$ 0.10	0.27 $\pm$ 0.04	0.41 (0.18 - 0.64)	0.0079
Muscle	0.57 $\pm$ 0.08	0.15 $\pm$ 0.02	0.42 (0.23 - 0.61)	0.0034
Lung	2.31 $\pm$ 0.02	0.83 $\pm$ 0.10	1.47 (1.24 - 1.70)	< 0.0001
Spleen	2.84 $\pm$ 0.02	1.63 $\pm$ 0.15	1.21 (0.87 - 1.56)	0.0006
Pancreas	0.67 $\pm$ 0.07	0.18 $\pm$ 0.02	0.49 (0.32 - 0.67)	0.0015
Kidney	12.39 $\pm$ 1.35	15.81 $\pm$ 1.89	-3.42 (-8.69 - 1.84)	0.1452
Stomach	3.23 $\pm$ 0.60	0.52 $\pm$ 0.11	2.71 (1.34 - 4.09)	0.0054
Intestines	2.78 $\pm$ 1.72	0.52 $\pm$ 0.09	3.57 (2.77 - 4.37)	0.0002
Liver	2.36 $\pm$ 0.36	1.15 $\pm$ 0.09	1.21 (0.37 - 2.05)	0.016
Femur	1.29 $\pm$ 0.22	0.38 $\pm$ 0.03	0.91 (0.41 - 1.42)	0.0075

Table S3 (a) Ex vivo biodistribution of wrists of mice administered [$^{68}\text{Ga}(\text{HP}_3\text{-RGD}_3)$], $n = 6$.

Group	Wrists 1 h PI, %ID ($\pm$ SEM)	Mean difference (95% CI) compared to animals with severe arthritis %ID	<i>p</i> value
Healthy	0.13 $\pm$ 0.010	0.11 (0.06 – 0.15)	0.0003
Mild Arthritis	0.15 $\pm$ 0.011	0.09 (0.05 – 0.14)	0.0013
Severe Arthritis	0.24 $\pm$ 0.020	-	-
Severe Arthritis with block	0.08 $\pm$ 0.005	0.16 (0.12 – 0.20)	< 0.0001
Organ/tissue	Wrists 1 h PI, %ID ($\pm$ SEM)	Mean difference (95% CI) compared to animals with mild arthritis %ID	<i>p</i> value
Healthy	0.13 $\pm$ 0.010	0.02 (-0.1 – 0.05)	0.2801

(b) Ex vivo biodistribution of ankles of mice administered [$^{68}\text{Ga}(\text{HP}_3\text{-RGD}_3)$], $n = 6$.

Group	Ankles 1 h PI, %ID ($\pm$ SEM)	Mean difference (95% CI) compared to animals with severe arthritis %ID	<i>p</i> value
Healthy	0.27 $\pm$ 0.010	0.35 (0.14 – 0.55)	0.0035
Mild Arthritis	0.30 $\pm$ 0.018	0.32 (0.11 – 0.53)	0.0061
Severe Arthritis	0.62 $\pm$ 0.100	-	-
Severe Arthritis with block	0.21 $\pm$ 0.028	0.41 (0.20 – 0.62)	0.0015
Organ/tissue	Ankles 1 h PI, %ID ($\pm$ SEM)	Mean difference (95% CI) compared to animals with mild arthritis %ID	<i>p</i> value
Healthy	0.27 $\pm$ 0.010	0.03 (0.01 – 0.07)	0.1682