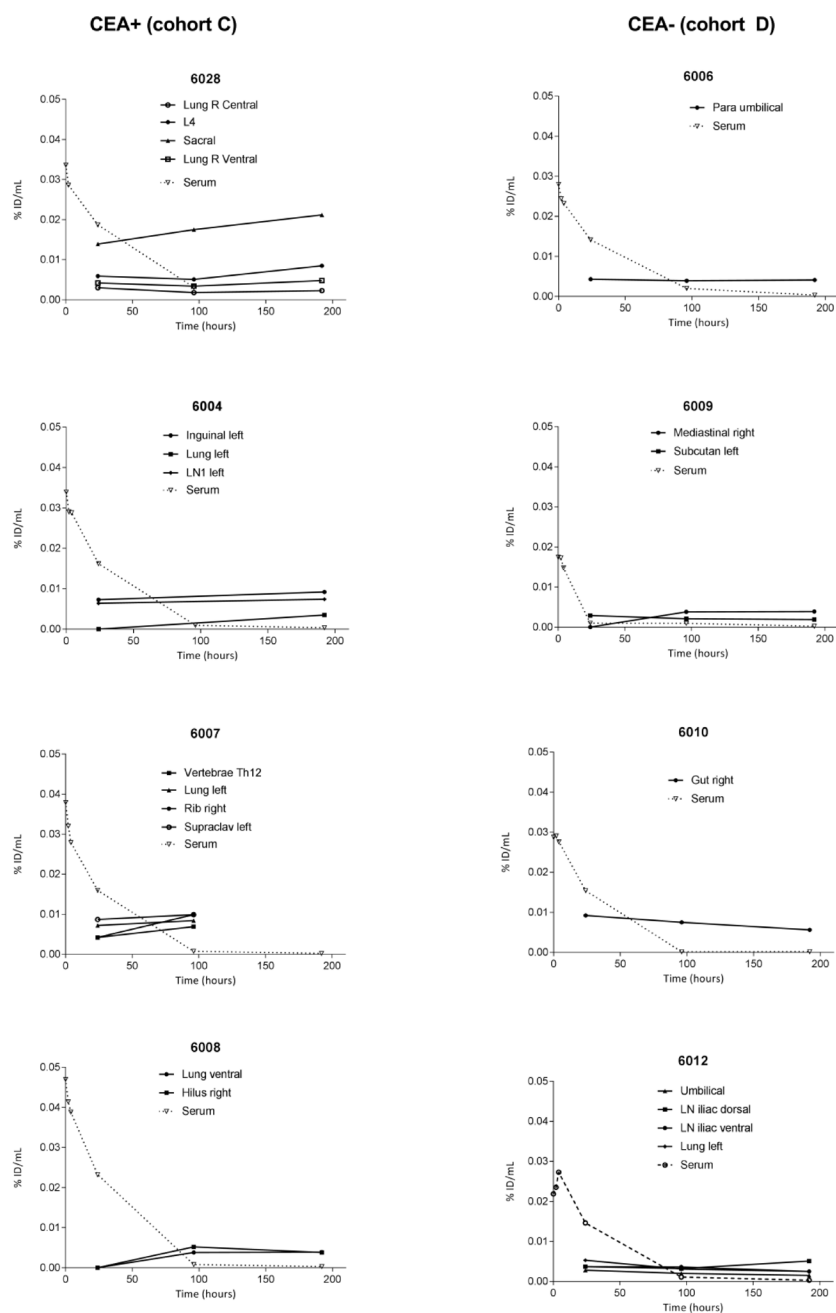
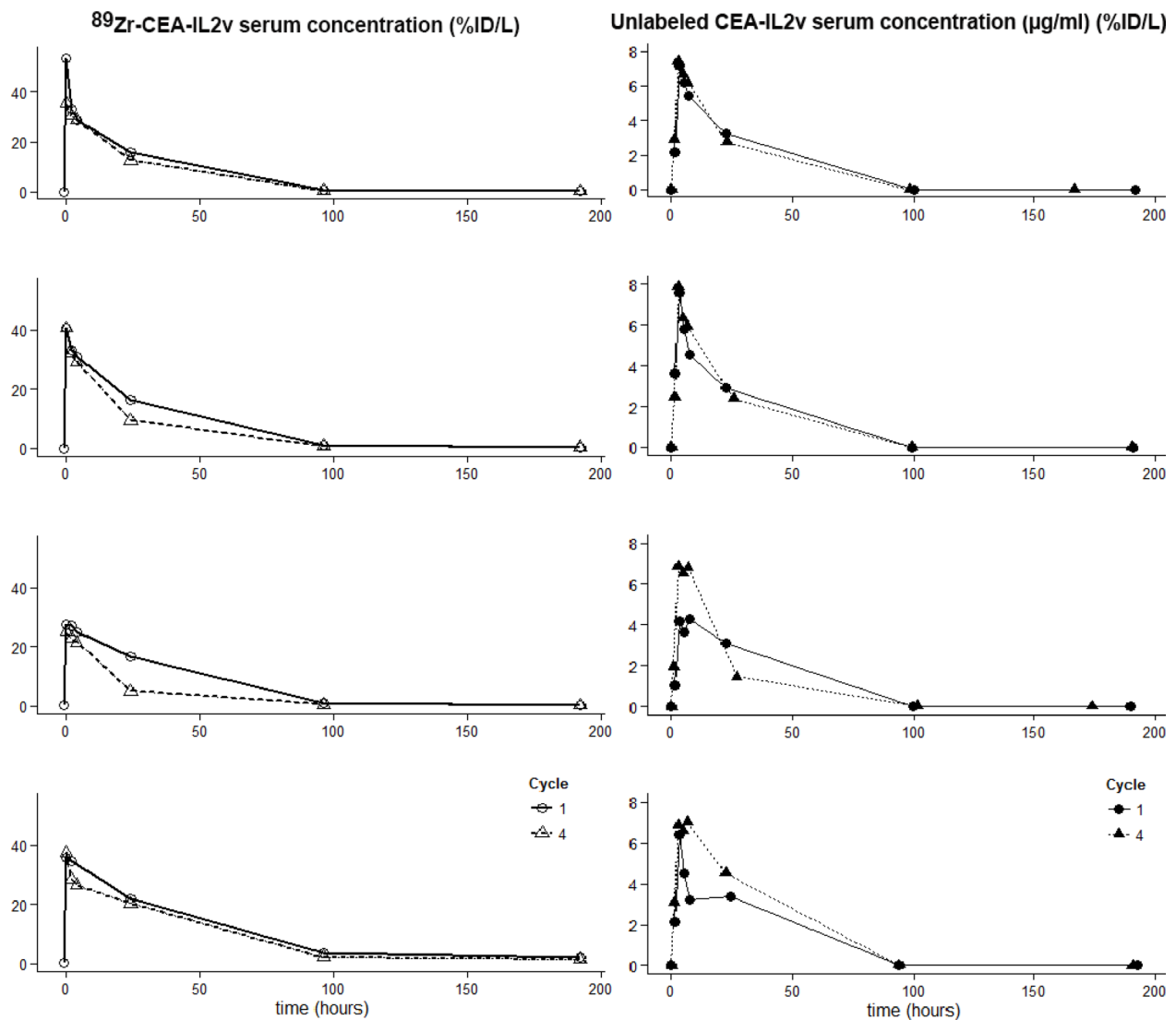


## <sup>89</sup>Zr-labeled CEA- targeted IL-2 variant immunocytokine in patients with solid tumors: CEA- mediated tumor accumulation and role of IL-2 receptor-binding

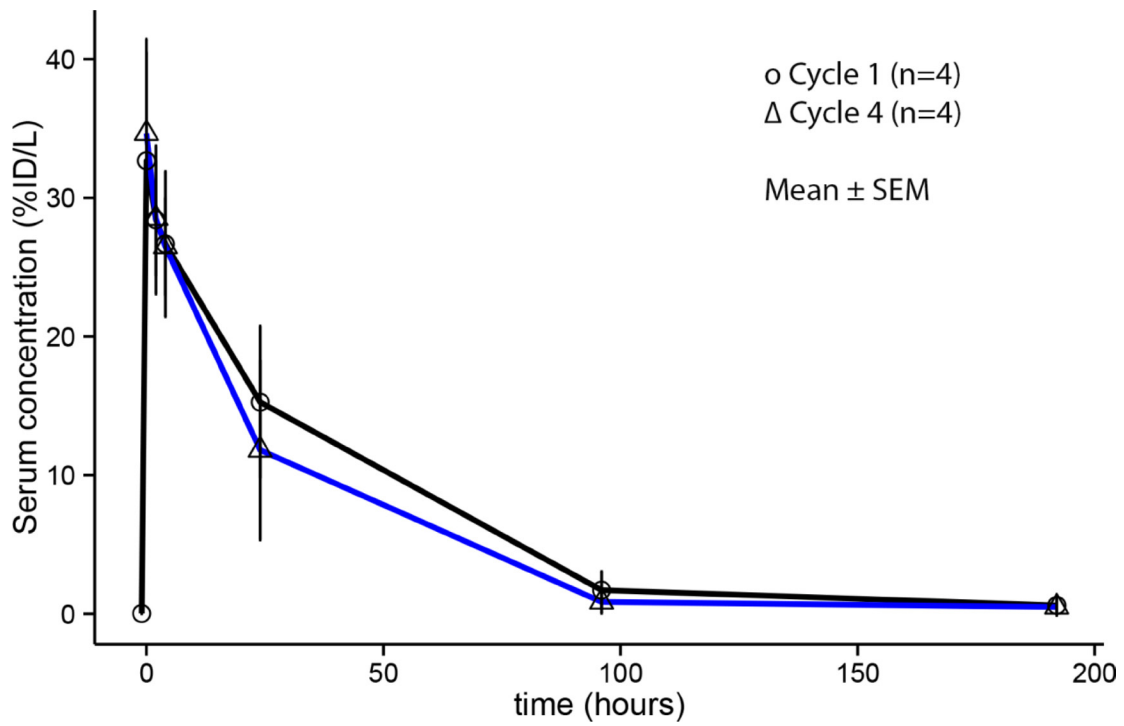
### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1:** <sup>89</sup>Zr-CEA- IL2v tumor uptake and serum levels of over time in CEA+ and CEA- patients treated with 30 mg. Data for individual patients from cohort C (CEA+; n = 4) and cohort D (CEA-; n = 4); tumor accumulation (solid lines) is %ID/mL<sub>peak</sub> and serum pools (dashed line) is %ID/mL.



**Supplementary Figure 2:  $^{89}\text{Zr}$ -CEA-IL2v and unlabeled CEA-IL2v exposure over time.** Individual serum concentration–time curves of  $^{89}\text{Zr}$ -CEA-IL2v in % injected dose/mL (%ID/mL; open symbols) and of unlabeled CEA-IL2v ( $\mu\text{g/mL}$ ) (filled symbols) in cycle 1 (solid line) vs. cycle 4 (dashed line) for four patients treated with 20 mg in the first q2w cycle and 30 mg thereafter (cohort E, CEA+ tumors). The patient represented in the third row had detectable anti-drug antibodies at cycle 4.



**Supplementary Figure 3:** <sup>89</sup>Zr-CEA-IL2v exposure at cycle 1 and cycle 4 in CEA+ patients from cohort E ( $n = 4$ ). Multiple drug administration induced faster clearance of CEA-IL2v, reducing drug exposure.

**Supplementary Table 1: Patient characteristics**

<b>Demographic characteristic</b>	<b>Value (N = 17)</b>
<b>Age (years) [range]</b>	65 [42–78]
<b>Gender (%)</b>	
<b>Male</b>	58%
<b>Female</b>	42%
<b>ECOG PS (%)</b>	
<b>0</b>	43%
<b>1</b>	57%
<b>Tumor type (n) (%)</b>	
<b>CEA+</b>	17
<b>CRC</b>	11 (46%)
<b>NSCLC</b>	4 (17%)
<b>Salivary gland</b>	1 (4.2%)
<b>Gastric</b>	1 (4.2%)
<b>CEA-</b>	7
<b>RCC</b>	3 (13%)
<b>Melanoma</b>	2 (8.3%)
<b>Ovarian</b>	1 (4.2%)
<b>Pancreatic cancer</b>	1 (4.2%)

ECOG PS = Eastern Cooperative Oncology Group Performance Status; CRC = colorectal carcinoma; NSCLC = non-small cell lung carcinoma; RCC = renal cell carcinoma.

**Supplementary Table 2: Tumor accumulation; assessment of <sup>89</sup>Zr-accumulation in <sup>18</sup>F-FDG+ lesions per patient**

Patient/ Cohort	Dose cycle 1 (mg)	CEA status	No. of FDG +ve lesions	No. of <sup>89</sup> Zr +ve lesions day 5	Accumulation visually confirmed	Consistency	
1/A	6	CEA+	7	1	Yes	Negative on day 2	
2/A	6	CEA+	5	1	Yes	Positive on day 2	
3/A	6	CEA+	5	0	No	Consistent lack of accumulation	
4/A	6	CEA+	11	7	Yes	Consistent	
5/B	6	CEA-	9	2	No	Both negative on day 1 and 2	
6/B	6	CEA-	6	0	No	Consistent lack of accumulation	
7/B	6	CEA-	7	0	No	Consistent lack of accumulation	
8/C	30	CEA+	7	5 <sup>a</sup>	Yes	Positive on day 2 and 9, day 5 scan not done	
9/C	30	CEA+	4	4	Yes	Consistent	
10/C	20	CEA+	8	2	Yes	Consistent	
11/ C	30	CEA+	10	6	Yes	Consistent	
12/C	30	CEA+	8	4	Yes	Positive on day 5 and 9.	
13/ D	30	CEA-	7	2	Yes	One lesion negative on day 2 (lymph node)	
14/D	30	CEA-	2	1	Yes	Consistent	
15/D	30	CEA-	8	1	Yes	Consistent	
16/D	30	CEA-	6	4	Yes	Consistent	
17/E	20	CEA+	4	0	No	Consistent lack of accumulation	
18/E	20	CEA+	33	27	Yes	Consistent	
19/E	20	CEA+	7	7	Yes	Consistent	
20/E	20	CEA+	2	Not evaluable	Not evaluable	Note: Lack of FDG+ extrahepatic lesions	
21/E	Cycle 1	20	CEA+	7	6	Yes	Consistent
	Cycle 4			7	4	Yes	Consistent
22/E	Cycle 1	20	CEA+	3	1	Yes	Consistent
	Cycle 4			3	1	Yes	Consistent
23/E	Cycle 1	20	CEA+	8	7	Yes	Consistent
	Cycle 4			8	3	Yes	Consistent
24/E	Cycle 1	20	CEA+	4	1	Yes	Consistent
	Cycle 4			4	1	Yes	Consistent

Accumulation was visually confirmed if <sup>89</sup>Zr-positive lesions were visual at the cycle 1, day 5 post-infusion plus one additional scan. <sup>a</sup>Primary evaluation at cycle 1, day 2 post-infusion because day 5 post-infusion scan was not performed.

**Supplementary Table 3: Biodistribution per dose cohort and by CEA status on cycle 1, day 5 post-infusion (non-tumor tissue)**

Organ (%ID/mL <sub>mean</sub> ±SD * 10 <sup>-2</sup> )	6 mg		20 mg <sup>a</sup>	30 mg		Total		Overall
	CEA+ (n = 4)	CEA- (n = 3)	CEA+ (n = 9)	CEA+ (n = 4)	CEA- (n = 4)	CEA+ (n = 17)	CEA- (n = 7)	
<b>Spleen</b>	1.4 ± 0.23	1.6 ± 0.51	1.2 ± 0.30	0.78 <sup>b</sup> ± 0.54	0.94 <sup>b</sup> ± 0.25	1.2 ± 0.40	1.2 ± 0.49	1.2 ± 0.42
<b>Liver</b>	1.1 ± 0.32	0.94 ± 0.22	1.2 ± 0.27	0.90 ± 0.65	1.1 ± 0.25	1.1 ± 0.39	1.01 ± 0.23	1.1 ± 0.35
<b>Vertebrae</b>	0.41 ± 0.11	0.46 ± 0.044	0.49 ± 0.15	0.40 ± 0.28	0.42 ± 0.086	0.45 ± 0.17	0.44 ± 0.068	0.44 ± 0.15
<b>Kidneys</b>	0.36 ± 0.058	0.39 ± 0.055	0.36 ± 0.070	0.26 ± 0.18	0.30 ± 0.015	0.34 ± 0.10	0.34 ± 0.059	0.34 ± 0.092
<b>Lung</b>	0.10 ± 0.010	0.10 ± 0.032	0.10 ± 0.030	0.072 ± 0.049	0.11 ± 0.024	0.10 ± 0.033	0.11 ± 0.026	0.10 ± 0.031

<sup>a</sup>Including the 20 mg (*n* = 1) and 20–30 mg cohort (*n* = 8).

<sup>b</sup>Significant difference (*p* < 0.05) assessed by One Way Analysis of Variance (ANOVA) comparing %ID/mL<sub>mean</sub> across 6 mg vs. 20 mg vs. 30 mg per organ and for CEA+ vs. CEA- per organ.