

Supplementary Materials of the Manuscript:

A novel tumor-specific agent for intraoperative near-infrared fluorescence imaging: a translational study in healthy volunteers and patients with ovarian cancer

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List of supplementary materials

Supplementary Materials and Methods

Questionnaire practical evaluation

Description of immunohistochemistry and fluorescence microscopy

Supplementary figures

Fig. S1. CRF Intraoperative detection ovarian cancer lesions

Fig. S2. Structure of OTL38

Fig. S3. CONSORT Flow diagram healthy volunteer study

Fig. S4. Pharmacokinetics in blood plasma of healthy volunteers and patients

Fig. S5. CONSORT Flow diagram ovarian cancer patient study

Fig. S6. TBR in ovarian cancer patients per dose group

Fig. S7. FR α and β staining of ovarian carcinoma tissue and a metastatic lymph node

Supplementary tables

Table S1. Listing of adverse events in the healthy volunteers study

Table S2. Summary of pharmacokinetic parameters

Table S3. TBR in ovarian cancer patients

Table S4. Listing of adverse events in the patient study

Table S5. Number and characteristics of lesions and lymph nodes identified with fluorescence

Other supplementary files

Data File S1. Exploratory evaluation of potential hypersensitivity responses to compound OTL38 utilizing human, *in vitro*, highly sensitive models

Movie S1. Intraoperative fluorescence detection and resection of a lymph node containing ovarian cancer metastases

Supplementary Material and Methods

Questionnaire practical evaluation:

Date:

Patient ID:

Questionnaire regarding the utility of OTL-038 and the imaging system

Please indicate to what extent you agree with the following statements:

	Totally disagree	Disagree	Neutral	Agree	Totally agree
1. The use of this technique represents added value to the surgical procedure					
2. You experienced discomfort during the surgical procedure by using the camera					
3. By using this technique you'll remove more tumor tissue and achieve more R0 res	0	0	0	0	0
4. If you weigh all the pros and cons of the investigated technique:					
a. You found this technique useful when performing surgery	0	0	0	0	0
b. You would like to use this technique more often in the future	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0

Description of immunohistochemistry and fluorescence microscopy:

The detection of FR α and FR β is determined by immunohistochemistry (IHC) in formalin-fixed, paraffin-embedded (FFPE) samples which are first deparaffinized with 3 changes of xylene and rehydrated in a series of alcohols (100%, 95%, then 70% EtOH) and finally rinsed well in running distilled water. Slides are then placed in a preheated DAKO Target Retrieval buffer for 40 minutes, then cooled in the buffer for 20 minutes followed by a 5 minute rinse in running distilled water. After the HIER (heat induced epitope retrieval) step, sections are incubated with 3% H₂O₂ in ethanol for 5 min. to inactivate the endogenous peroxides followed by a Protein Blocking step for 5 minutes. Slides are rinsed well with TBST wash buffer.

- For FR β : Sections are incubated for 30 minutes at room temperature with the biotinylated- human anti-human FR β at a 1:100 dilution. Sections are rinsed with TBST wash buffer. SAV-Px (Thermo Scientific) is applied to the sections and incubated for 10 minutes followed with TBST wash buffer.
- For FR α : Sections are incubated for 30 minutes at room temperature with with Folate Receptor alpha antibody. After a 10 minute incubation with MACH 4 Mouse Probe, sections were incubated with MACH 4 HRP Polymer.

Sections should then be incubated in 3,3'-diaminobenzidine (DAB+) 5 minutes, counterstained with Modified Schmidt's Hematoxylin for 5 minutes followed by a 3 minute tap water rinse to blue sections, dehydrated through graded alcohols and cleared in 3 changes of xylene and mounted with a permanent mounting media.

For fluorescence microscopy frozen or paraffin embeded sections of 5 μ m were mounted with Prolong Gold with DAPI (Life Technologies). Fluorescent signals were detected using a

Leica DM5500B fluorescence microscope. To detect DAPI and OTL38 fluorescence, we used the following filter set:

- DAPI filter set for detecting DAPI (blue color): excitation peak at 358 nm, emission spectrum is broad and peaks at 461 nm.
- Cy7 filter set for detecting OTL38 (green color): excitation peak at 750 nm, emission peak at 773 nm.

A range of 30 nm above and below the emission and excitation peaks can be excited and detected.

Supplementary figures

Fig. S1. CRF Intraoperative detection ovarian cancer lesions

PHASE I CLINICAL STUDY **PRE/POST-SURGERY** Surgical Reporting Form

Patient Study ID No.			Recorded by			Date		
OVARIAN TUMOR/ LESION DETECTION CHART								
						<ul style="list-style-type: none"> A- Appendix AC- Ascending Colon ACS- Anterior Cul de Sac B- Bladder Cec- Cecum Cer- Cervix D- Duodenum DC- Descending Colon GB- Gallbladder GO- Greater Omentum H- Heart I- Ileum J- Jejunum LD- Left Diaphragm LFT- Left Fallopian Tube LK- Left Kidney LL- Left Lung LLL- Left Lobe of the Liver LO- Lesser Omentum LOV- Left Ovary LPG- Left Paracolic Gutter LU- Left Ureter P- Pancreas PPP- Posterior Pelvic Peritoneum R- Rectum RD- Right Diaphragm RFT- Right Fallopian Tube RK- Right Kidney RL- Right Lung RLL- Right Lobe of the Liver ROV- Right Ovary RPG- Right Paracolic Gutter RU- Right Ureter SC- Sigmoid Colon Sp- Spleen St- Stomach TC- Transverse Colon U- Uterus V- Vagina 		
TUMOR DETECTION			INDICATE TUMOR TYPE					
			Single Tumor			Diffuse Tumor		
Fluorescence Only			P			MIL P		
If NO ADDITIONAL LESIONS OBSERVED check box to right								

Mesenteric tumors may be indicated with the appropriate symbol from above and an 'MT'.
 MIL if lesions are too numerous to count in a specific region/organ.

Case report form (CRF) used to mark the location of a lesion found during surgery, including presence of fluorescence (marked with X), clinically suspect for malignancy (marked with O) or both (marked with X in O)

Fig. S2. Structure of OTL38

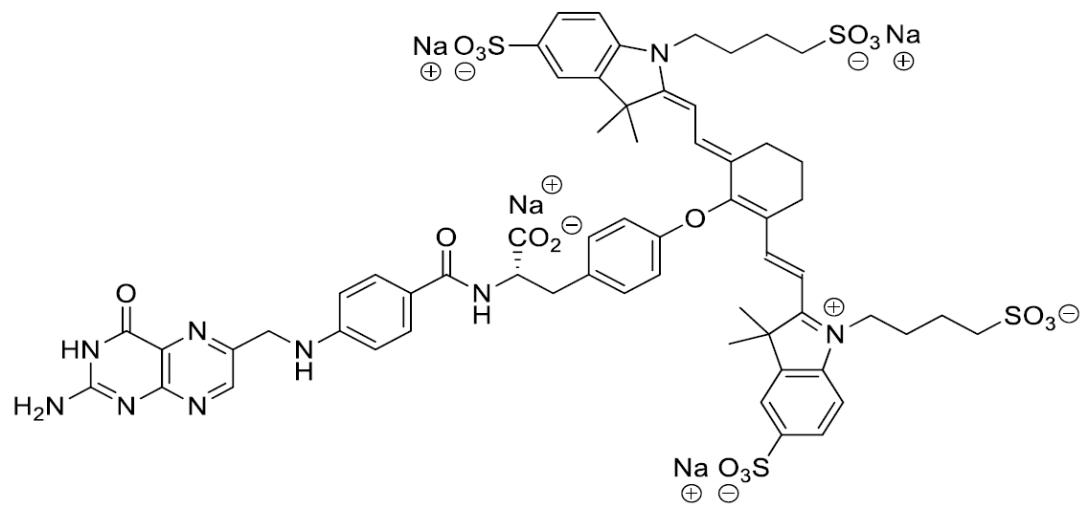


Fig. S3. CONSORT Flow diagram healthy volunteer study

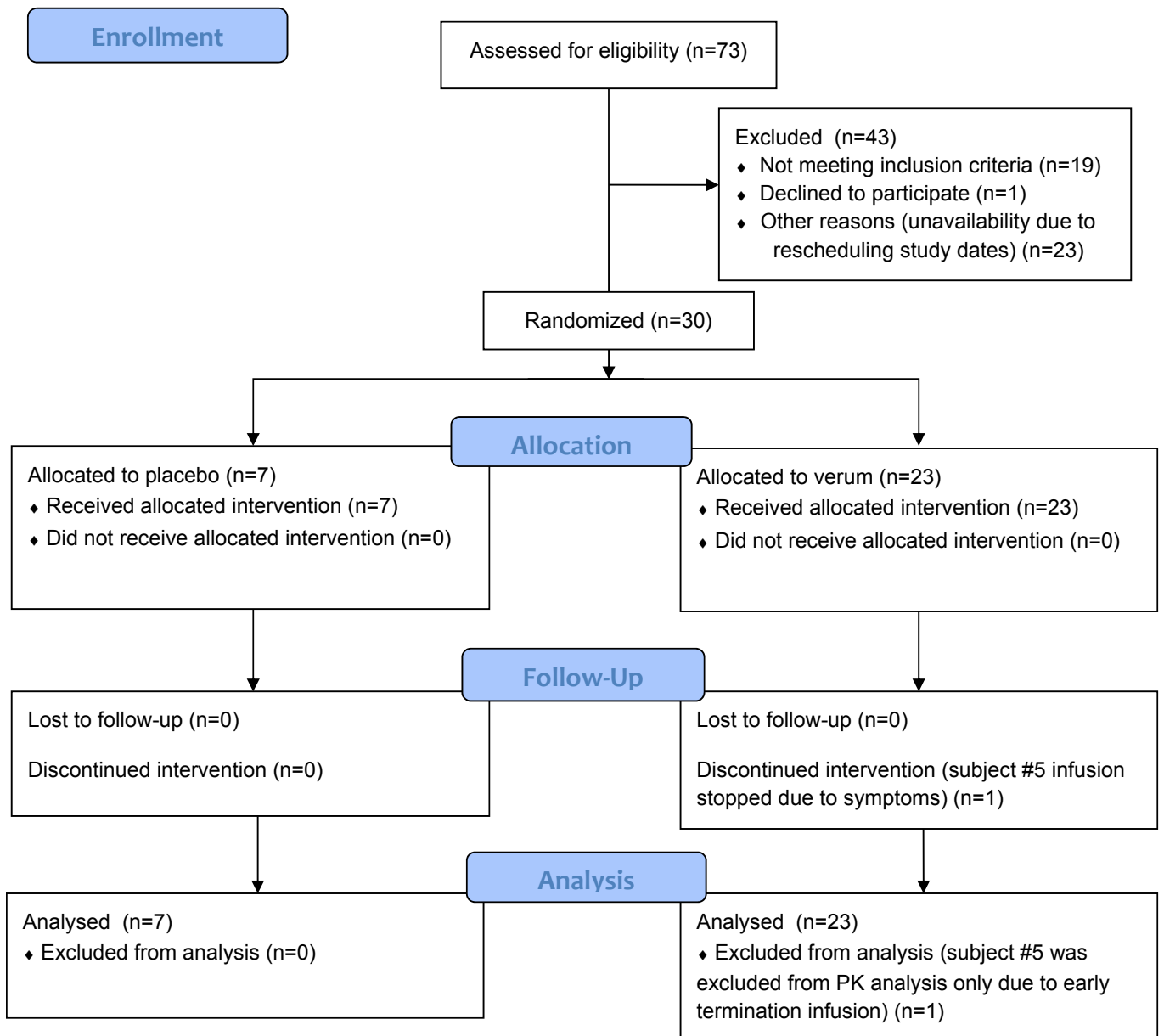
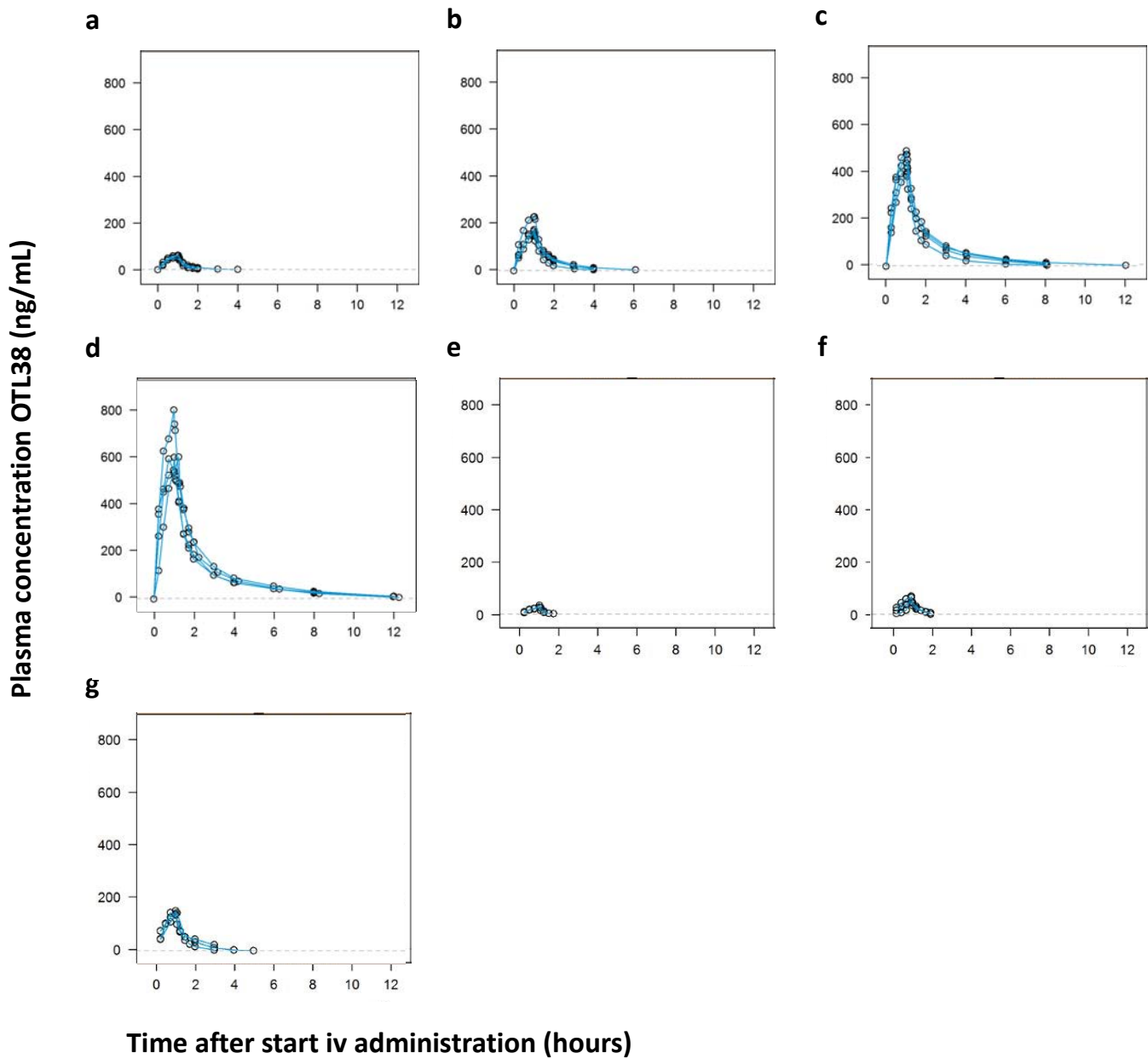


Fig. S4. Pharmacokinetics in blood plasma of healthy volunteers and patients



a-g OTL38 plasma concentration (ng/mL) over time (hours). With on the upper row the different doses (**a** 0,025mg/kg, **b** 0.05mg/kg, **c** 0.1mg/kg, **d** 0.2mg/kg) in healthy volunteers and the lower row the different doses (**e** 0.0125mg/kg, **f** 0.025mg/kg, **g** 0.05mg/kg) in patients with ovarian cancer.

Fig. S5. CONSORT Flow diagram ovarian cancer patient study

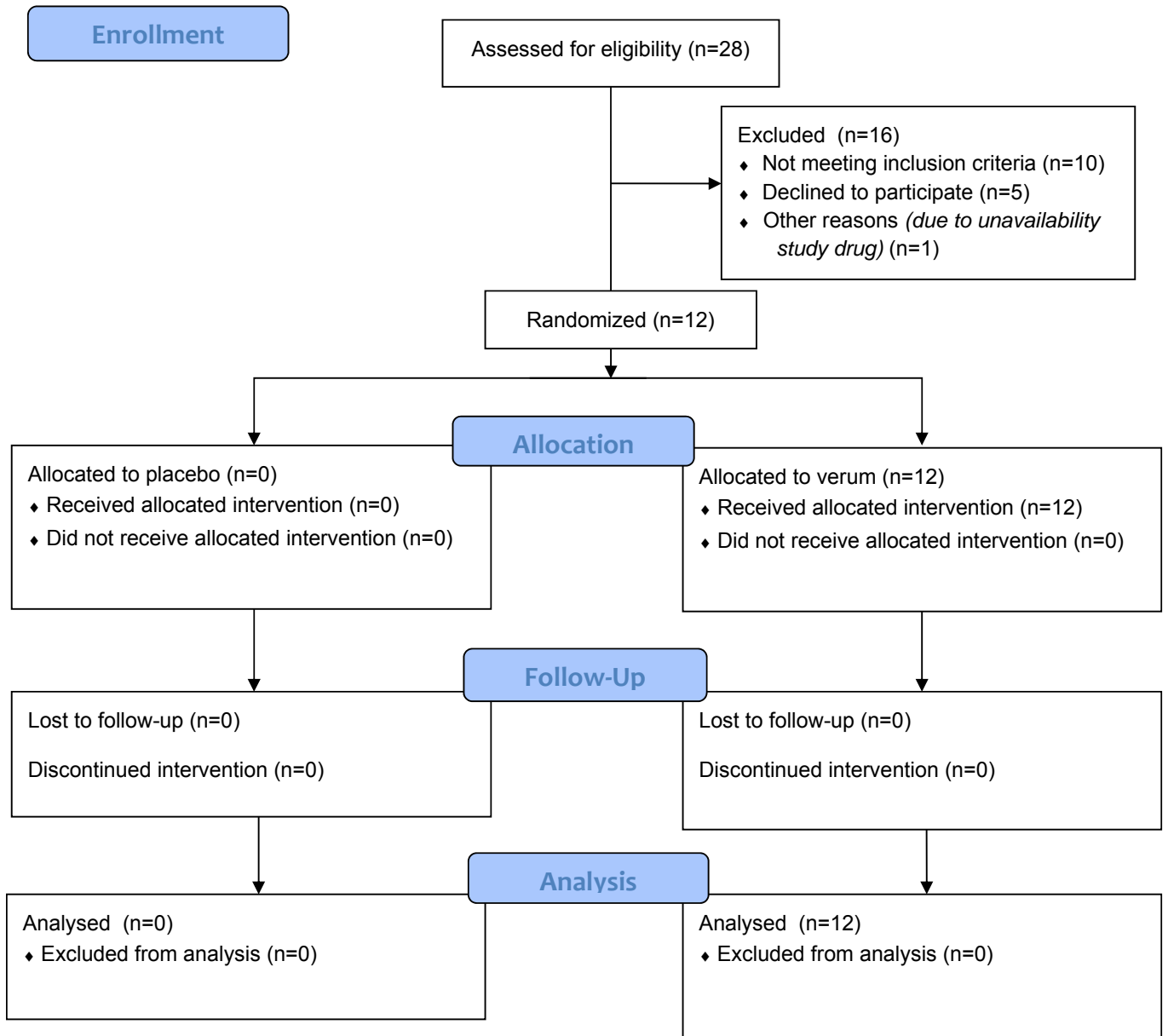


Fig. S6. TBR in ovarian cancer patients per dose group

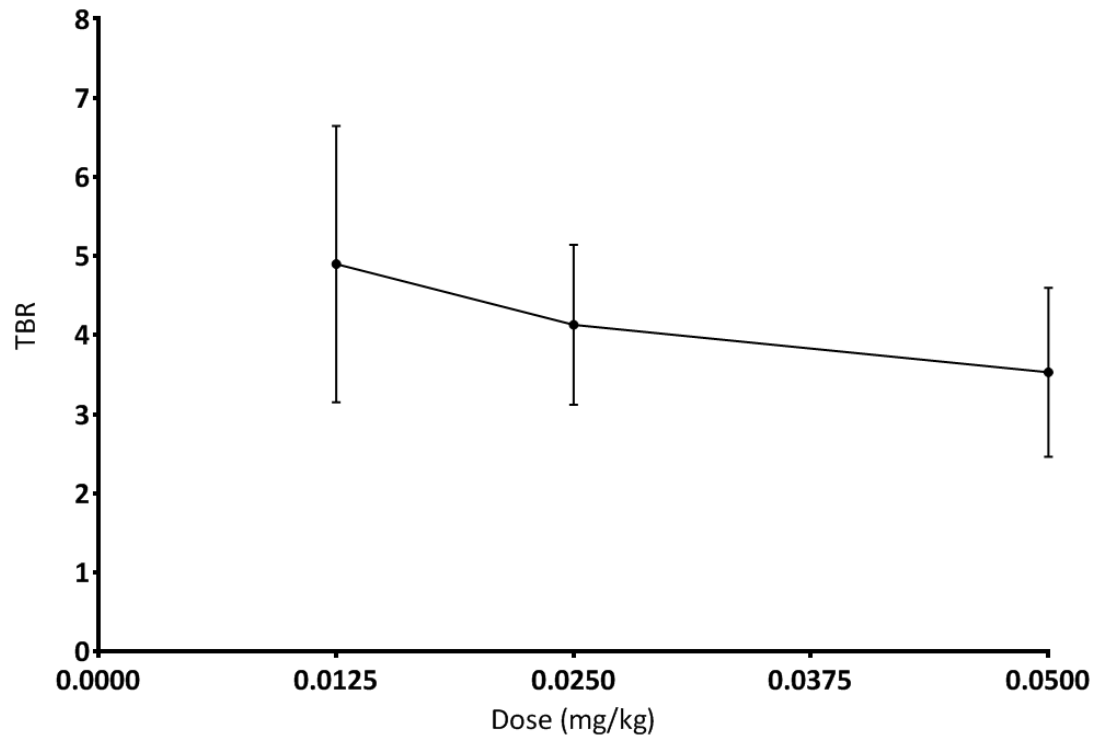
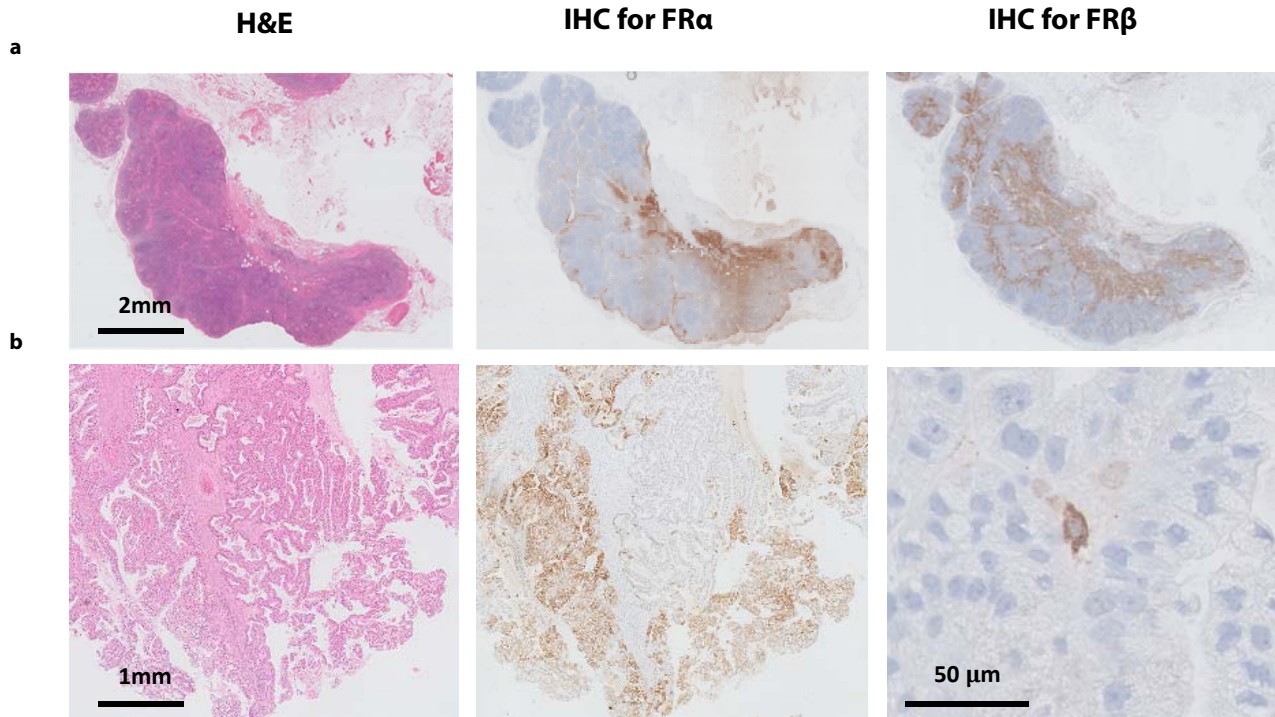


Fig. S7. FR α and β staining of ovarian carcinoma tissue and a metastatic lymph node



The images show a lymph node containing ovarian cancer metastases on hematoxylin and eosin (H&E) staining (upper left panel) and was positive for both FR α and FR β immunostaining (upper middle and right panel). Immunostaining of the primary tumor showed a positive staining for FR α (bottom middle panel), which corresponds to the anatomical site containing a serous ovarian adenocarcinoma visible on hematoxylin and eosin (H&E) staining (bottom left panel), immunostaining for FR β showed positive staining activated macrophages between negative staining tumor cells.

Supplementary Tables

Table S1. Listing of Adverse Events Healthy in the Volunteer Study

Listing of adverse events

(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
1	0.025_OTL038S (0.9% NaCl, 10 min)	V1	13JAN2014 10:30/ 0D00H04M	13JAN2014 10:34	0D00H41M	ABDOMINAL DISCOMFORT	Abdominal discomfort	None	single occasion	moderate	Related	No
			13JAN2014 10:30/ 0D00H15M	13JAN2014 10:45	0D00H30M	PRESYNCOPE	pale and sweaty	No	single occasion	mild	Related	No
			13JAN2014 10:30/ 0D00H20M	13JAN2014 10:50	0D04H10M	ORAL PRURITUS	Tingling feeling throat	No	single occasion	mild	Related	No
			13JAN2014 10:30/ 0D01H30M	13JAN2014 12:00	0D01H00M	URTICARIA	Urticaria and itching on arms , face and torso	Tavegyl (clemast ine) 1mg in 1ml i.v.	single occasion	moderate	Related	No
			13JAN2014 10:30/ 0D20H30M	14JAN2014 7:00	10D00H00M	CATHETER SITE PHLEBITIS	Redness at canula's injection site on arms	No	single occasion	mild	unrelated	No
			13JAN2014 10:30/ 0D00H04M	13JAN2014 10:34	0D00H41M	NAUSEA	nausea	no	single occasion	moderate	Related	No
3	0.025_OTL038S (0.9% NaCl, 60 min)	V1	04FEB2014 9:00/ 0D01H00M	04FEB2014 10:00	0D00H29M	DIZZINESS	lightheaded	No	single occasion	mild	probable	No
			04FEB2014 9:00/ 0D01H35M	04FEB2014 10:35	0D00H15M	DISTURBANCE IN ATTENTION	less alert	No	single occasion	mild	possible	No
4	0.025_OTL038S (0.9% NaCl, 60 min)	V1	04FEB2014 10:07/ 0D12H19M	04FEB2014 22:26	0D00H34M	INJECTION SITE NODULE	small nodus around insertion place of cannula	no	single occasion	mild	unrelated	No
5	0.025_OTL038S (0.9% NaCl, 60 min)	V1	05FEB2014 9:00/ 0D00H07M	05FEB2014 9:07	0D00H13M	ABDOMINAL DISCOMFORT	stomach ache	none	single occasion	moderate	Related	No

Note: M=Missing; NA=Not Applicable; SCR=Screening

Listing of adverse events
(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
5	0.025_OTL038S (0.9% NaCl, 60 min)	V1	05FEB2014 9:00/	05FEB2014 0D00H08M	0D00H12M	NAUSEA 9:08	nausea	none	single	moderate	Related occasion	No
			05FEB2014 9:00/ 0D00H09M	05FEB2014 9:09	0D00H03M	VOMITING	vomiting	infusion OTL-038 stopped	single occasion	moderate	Related	No
7	0.05 OTL-038 Glucose	V1	15APR2014 11:49/ 0D01H16M	15APR2014 13:05	0D00H15M	DIZZINESS	Lightheaded	no	single occasion	mild	probable	No
9	0.05 OTL-038 Glucose	V1	15APR2014 10:41/ 0D00H24M	15APR2014 11:05	0D03H40M	SOMNOLENCE	sleepy	no	single occasion	mild	probable	No
11	0.05 OTL-038 Glucose	V1	16APR2014 10:17/ 0D00H08M	16APR2014 10:25	0D00H27M	GINGIVAL PRURITUS	tingling feeling gums	no	single occasion	mild	Related	No
12	Placebo	V1	22APR2014 10:17/ 0D04H43M	22APR2014 15:00	0D02H30M	FATIGUE	tired	no	single occasion	mild	possible	No
13	0.1 OTL-038 Glucose	V1	22APR2014 11:24/ predose	22APR2014 11:10	M	DIZZINESS	dizzy	no	intermittent	mild	unlikely	No
			22APR2014 11:24/ 0D00H06M	22APR2014 11:30	0D00H15M	NAUSEA	nauseous	no	single occasion	mild	Related	No
			22APR2014 11:24/ 0D00H07M	22APR2014 11:31	0D00H19M	ORAL PRURITUS	itch in mouth	no	single occasion	mild	Related	No
			22APR2014 11:24/ 0D00H16M	22APR2014 11:40	0D00H05M	SENSATION OF HEAVINESS	heavy eyelids	no	single occasion	mild	possible	No

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Listing of adverse events
(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
13	0.1 OTL-038 Glucose	V1	22APR2014 11:24/ 0D00H16M	22APR2014 11:40	0D00H10M	DYSPNOEA	breathing feel heavier than usual	No action taken, saturati on was 100% and subject was not wheezing	single occasion	mild	Related	No
			22APR2014 11:24/ 0D00H21M	22APR2014 11:45	0D01H15M	NASAL CONGESTION	stuffed nose	no	single occasion	mild	Related	No
			22APR2014 11:24/ 0D01H04M	22APR2014 12:28	0D02H32M	ABDOMINAL DISCOMFORT	stomach ache	no	single occasion	mild	probable	No
			22APR2014 11:24/ 0D01H21M	22APR2014 12:45	0D00H45M	NERVOUSNESS	shaky	no	single occasion	mild	possible	No
			22APR2014 11:24/ 0D04H36M	22APR2014 16:00	0D04H00M	FATIGUE	tired	no	single occasion	mild	probable	No
			22APR2014 11:24/ 0D04H36M	22APR2014 16:00	0D04H00M	HEADACHE	headache	no	single occasion	mild	possible	No
			22APR2014 11:24/ 1D05H06M	23APR2014 16:30	0D02H30M	EAR DISCOMFORT	pressure in right ear	no	single occasion	mild	unlikely	No
			14	0.1 OTL-038 Glucose	V1	22APR2014 12:31/ 0D00H02M	22APR2014 12:33	0D00H57M	ORAL PRURITUS	tickle in throat	no	single occasion
22APR2014 12:31/ 0D00H04M	22APR2014 12:35	0D01H31M				HEAD DISCOMFORT	fullness in head	no	single occasion	mild	Related	No
15	0.1 OTL-038 Glucose	V1	23APR2014 8:55/ 0D00H08M	23APR2014 9:03	0D00H00M	SNEEZING	sneezing	no	single occasion	mild	Related	No
			23APR2014 8:55/ 0D00H08M	23APR2014 9:03	0D00H10M	RHINITIS ALLERGIC	itchy feeling nose	no	single occasion	mild	Related	No

Note: M=Missing; NA=Not Applicable; SCR=Screening

Listing of adverse events
(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
15	0.1 OTL-038 Glucose	V1	23APR2014 8:55/ 0D08H04M	23APR2014 16:59	0D00H11M	DIZZINESS	feeling dizzy due to cannula problems	no	single occasion	mild	unrelated	No
			23APR2014 8:55/ 1D05H05M	24APR2014 14:00	0D18H00M	BODY TEMPERATURE INCREASED	fever, temp 38.8, no other symptoms	no	single occasion	mild	unlikely	No
16	0.1 OTL-038 Glucose	V1	23APR2014 10:02/ 0D00H03M	23APR2014 10:05	0D00H24M	NAUSEA	nausea	no	single occasion	mild	Related	No
			23APR2014 10:02/ 0D00H03M	23APR2014 10:05	0D00H14M	PRURITUS ALLERGIC	tingling tongue lips throat and nose	no	single occasion	mild	Related	No
			23APR2014 10:02/ 0D00H05M	23APR2014 10:07	0D00H13M	FEELING HOT	feeling warm inside	no	single occasion	mild	probable	No
			23APR2014 10:02/ 0D00H17M	23APR2014 10:19	0D00H56M	DRY MOUTH	dry mouth	no	single occasion	mild	probable	No
			23APR2014 10:02/ 0D00H21M	23APR2014 10:23	0D00H52M	PARAESTHESIA	tingling right hand	no	single occasion	mild	probable	No
			23APR2014 10:02/ 0D02H58M	23APR2014 13:00	1D02H00M	SOMNOLENCE	drowsiness and sleepy	no	single occasion	mild	possible	No
			23APR2014 10:02/ 0D08H08M	23APR2014 18:10	0D00H05M	DIZZINESS	dizzy	no	single occasion	mild	possible	No
			23APR2014 10:02/ 0D11H28M	23APR2014 21:30	0D09H45M	HEADACHE	headache	no	single occasion	mild	possible	No
			23APR2014 10:02/ 0D21H13M	24APR2014 7:15	0D01H45M	CHILLS	cold, shivering	no	single occasion	mild	unlikely	No
			23APR2014 10:02/ 0D11H58M	23APR2014 M 22:00		INJECTION SITE HAEMATOMA	hematoma from cannula	no	single occasion	mild	unrelated	No
			17	0.2 OTL-038 Glucose	V1	29APR2014 9:11/ 0D00H01M	29APR2014 9:12	0D00H14M	NAUSEA	nausea	no	single occasion
29APR2014 9:11/ 0D00H01M	29APR2014 9:12	0D00H03M				VOMITING	vomiting	no	single occasion	moderate	Related	No

Note: M=Missing; NA=Not Applicable; SCR=Screening

Listing of adverse events
(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
17	0.2 OTL-038 Glucose	V1	29APR2014 9:11/ 0D00H03M	29APR2014 9:14	0D00H55M	FEELING HOT	feeling hot	no	single occasion	mild	probable	No
			29APR2014 9:11/ 0D00H01M	29APR2014 9:12	0D00H14M	PRESYNCOPE	light headed	no	single occasion	mild	Related	No
			29APR2014 9:11/ 0D00H05M	29APR2014 9:16	0D00H10M	GINGIVAL PRURITUS	itch gums	no	single occasion	mild	Related	No
			29APR2014 9:11/ 0D00H09M	29APR2014 9:20	0D00H08M	PARAESTHESIA	tingling feeling feet	no	single occasion	mild	probable	No
			29APR2014 9:11/ 0D00H15M	29APR2014 9:26	0D01H44M	HEAD DISCOMFORT	heavy head, pressure	no	single occasion	mild	Related	No
			18	0.2 OTL-038 Glucose	V1	29APR2014 10:18/ 0D00H02M	29APR2014 10:20	0D00H27M	NAUSEA	nausea	infusion stopped temporar ily (15 minutes)	intermittent
29APR2014 10:18/ 0D00H02M	29APR2014 10:20	0D00H27M				ABDOMINAL DISCOMFORT	stomach ache	infusion stopped temorari ly (15 minutes)	single occasion	moderate	Related	No
29APR2014 10:18/ 0D00H02M	29APR2014 10:20	0D00H55M				ORAL PRURITUS	throat itch	no	single occasion	mild	Related	No
29APR2014 10:18/ 0D00H02M	29APR2014 10:20	0D00H55M				EAR PAIN	ear pain/pressure	no	single occasion	mild	probable	No
29APR2014 10:18/ 0D00H07M	29APR2014 10:25	0D00H50M				DIZZINESS	dizzy	infusion stopped temporar ily (15 minutes)	intermittent	moderate	Related	No
29APR2014 10:18/ 0D00H47M	29APR2014 11:05	0D00H40M				FLANK PAIN	side stich right	no	single occasion	mild	probable	No

Note: M=Missing; NA=Not Applicable; SCR=Screening

Listing of adverse events
(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
18	0.2 OTL-038 Glucose	V1	29APR2014 10:18/ 0D00H02M	29APR2014 10:20	0D00H55M	ALLERGIC COUGH	dry cough	no	intermittent	mild	probable	No
20	0.2 OTL-038 Glucose	V1	30APR2014 9:06/ 0D00H02M	30APR2014 9:08	0D00H05M	NAUSEA	nausea	tavegyl 2mg administ ered iv	single occasion	moderate	Related	No
			30APR2014 9:06/ 0D00H06M	30APR2014 9:12	0D00H18M	HEAD DISCOMFORT	headache pressing	no	single occasion	mild	Related	No
			30APR2014 9:06/ 0D00H09M	30APR2014 9:15	0D08H45M	SOMNOLENCE	sleepy	no	single occasion	mild	unlikely	No
21	0.2 OTL-038 Glucose	V1	30APR2014 10:18/ 0D00H02M	30APR2014 10:20	0D00H10M	NAUSEA	nausea	no	single occasion	mild	Related	No
			30APR2014 10:18/ 0D00H05M	30APR2014 10:23	0D00H08M	PAIN IN JAW	pressure pain jaw	no	single occasion	mild	probable	No
			30APR2014 10:18/ 0D00H08M	30APR2014 10:26	0D00H05M	ORAL PRURITUS	strange feeling mouth	no	single occasion	mild	Related	No
			30APR2014 10:18/ 0D00H10M	30APR2014 10:28	0D01H32M	URTICARIA	urticaria face	tavegyl 1mg iv administ ered	single occasion	mild	Related	No
			30APR2014 10:18/ 0D00H15M	30APR2014 10:33	0D04H12M	SOMNOLENCE	sleepy	no	single occasion	mild	unlikely	No
22	0.025 OTL-038 Glucose	V1	08APR2014 9:20/ 0D00H10M	08APR2014 9:30	0D00H30M	ABDOMINAL DISCOMFORT	stomach ache	no	single occasion	mild	Related	No
			08APR2014 9:20/ 0D02H00M	08APR2014 11:20	0D05H40M	SOMNOLENCE	feeling sleepy	no	single occasion	mild	probable	No
23	0.025 OTL-038 Glucose	V1	08APR2014 10:32/ 0D00H03M	08APR2014 10:35	0D01H40M	NAUSEA	nausea	no	single occasion	mild	Related	No

Note: M=Missing; NA=Not Applicable; SCR=Screening

Listing of adverse events
(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
23	0.025 OTL-038 Glucose	V1	08APR2014 10:32/ 0D00H06M	08APR2014 10:38	0D00H10M	ORAL PRURITUS	burning sensation in the throat	no	single occasion	mild	Related	No
			08APR2014 10:32/ 0D00H21M	08APR2014 10:53	0D01H07M	BUCCAL MUCOSAL ROUGHENING	surface palate feel rougher than normal	no	single occasion	mild	probable	No
			08APR2014 10:32/ 0D11H33M	08APR2014 22:05	0D00H25M	HEADACHE	headache	no	single occasion	mild	possible	No
			08APR2014 10:32/ 1D23H37M	10APR2014 M 10:09		TRAUMATIC HAEMATOMA	small bruise caused by tourniquet	no	single occasion	mild	unrelated	No
24	0.025 OTL-038 Glucose	V1	08APR2014 11:44/ 0D00H19M	08APR2014 12:03	1D11H42M	SOMNOLENCE	sleepiness	no	single occasion	mild	probable	No
25	0.025 OTL-038 Glucose	V1	09APR2014 9:20/ 0D00H15M	09APR2014 9:35	0D03H25M	SOMNOLENCE	sleepy	no	single occasion	mild	probable	No
			09APR2014 9:20/ 1D02H40M	10APR2014 12:00	0D01H30M	HEADACHE	headache	no	single occasion	mild	unlikely	No
27	0.2 OTL-038 Gluc + preMed	V1	23JUN2014 11:40/ 0D00H05M	23JUN2014 11:45	0D00H15M	SOMNOLENCE	drowsiness	no	single occasion	mild	possible	No
			23JUN2014 11:40/ 0D00H05M	23JUN2014 11:45	0D00H13M	ABDOMINAL DISCOMFORT	strange feeling abdomen	no	single occasion	mild	Related	No
28	0.2 OTL-038 Gluc + preMed	V1	23JUN2014 10:40/ 0D00H03M	23JUN2014 10:43	0D00H22M	NAUSEA	nausea	no	single occasion	mild	Related	No
			23JUN2014 10:40/ 0D00H04M	23JUN2014 10:44	0D00H21M	FEELING HOT	feeling warm	no	single occasion	mild	probable	No
			23JUN2014 10:40/ 0D00H05M	23JUN2014 10:45	0D00H20M	ABDOMINAL DISCOMFORT	abdominal pain	no	single occasion	mild	Related	No
			23JUN2014 10:40/ 0D00H13M	23JUN2014 10:53	0D00H12M	ORAL PRURITUS	itch in mouth	no	single occasion	mild	Related	No

Note: M=Missing; NA=Not Applicable; SCR=Screening

Listing of adverse events
(Safety Population)

Subject	Treatment	Visit	Dosing Date-Time/ Time from Admin	Start Date/Time	Duration (DDHHMM)	Diagnosis	Signs and Symptoms	Action	Outcome	Severity	Treatment related	SAE
28	0.2 OTL-038 Gluc + preMed	V1	23JUN2014 10:40/ 1D05H20M	24JUN2014 16:00	0D01H00M	FATIGUE	fatigue	no	single occasion	mild	unlikely	No
30	0.2 OTL-038 Gluc + preMed	SCR	NA/ M	22JUN2014 22:00	1D11H00M	PHARYNGITIS	sore throat	no	single occasion	mild	unrelated	No
		V1	24JUN2014 10:27/ 0D00H04M	24JUN2014 10:31	0D00H14M	ABDOMINAL DISCOMFORT	abdominal pain	no	single occasion	mild	Related	No
			24JUN2014 10:27/ 0D00H04M	24JUN2014 10:31	0D00H14M	NAUSEA	nausea	no	single occasion	mild	Related	No

Table S2. Summary of pharmacokinetic parameters per treatment

Summary PK parameters of 0.025_OTL038 (in 0.9% NaCl)

Parameter	min	max	mean	median	sd
Cmax (ng/ml)	5.04	150	71.2	64.9	59.7
tmax (hr)	0.250	1.00	0.625	0.625	0.433
AUC_0_last (ng*hr/ml)	0.630	75.1	47.7	57.5	32.4
AUMC_0_last (ng*hr ² /ml)	0.158	51.3	35.6	45.5	24.2

Summary PK parameters of 0.025_OTL038

Parameter	min	max	mean	median	sd
Cmax (ng/ml)	50.3	62.9	59.1	61.7	5.94
tmax (hr)	0.750	1.03	0.954	1.02	0.137
AUC_0_last (ng*hr/ml)	49.6	78.2	61.6	59.2	12.4
AUMC_0_last (ng*hr ² /ml)	41.1	85.8	57.3	51.1	19.7

Summary PK parameters of 0.05_OTL038

Parameter	min	max	mean	median	sd
Cmax (ng/ml)	159	231	184	173	32.2
tmax (hr)	1.02	1.05	1.03	1.02	0.0160
AUC_0_last (ng*hr/ml)	189	309	250	250	53.7
AUMC_0_last (ng*hr ² /ml)	208	444	329	332	100

Summary PK parameters of 0.1_OTL038

Parameter	min	max	mean	median	sd
Cmax (ng/ml)	390	493	450	458	45.7
tmax (hr)	1.00	1.03	1.02	1.02	0.0136
AUC_0_last (ng*hr/ml)	573	905	785	830	157
AUMC_0_last (ng*hr ² /ml)	908	2060	1520	1560	486

Summary PK parameters of 0.2_OTL038

Parameter	min	max	mean	median	sd
Cmax (ng/ml)	542	807	640	606	115
tmax (hr)	1.02	1.25	1.09	1.04	0.109
AUC_0_last (ng*hr/ml)	1170	1570	1310	1250	178
AUMC_0_last (ng*hr ² /ml)	2870	3800	3250	3150	395

AUC_0_inf (ng*hr/ml)	1200	1610	1340	1280	180
Lambda_z (hr-1)	0.215	0.310	0.265	0.267	0.0391
Term (hr)	2.23	3.22	2.66	2.60	0.410
PercAUCEstrap (%)	1.70	4.21	2.80	2.65	1.05
CL_F (L/hr)	9.00	11.2	10.0	9.98	0.973
V_F (L)	30.6	51.9	38.8	36.4	9.24

Summary PK parameters of 0.2_OTL038 (after premedication with clemastine)

Parameter	min	max	mean	median	sd
Cmax (ng/ml)	636	874	765	784	120
tmax (hr)	1.00	1.00	1.00	1.00	0
AUC_0_last (ng*hr/ml)	1180	1540	1400	1490	194
AUMC_0_last (ng*hr ² /ml)	2770	3650	3230	3260	439
AUC_0_inf (ng*hr/ml)	1220	1550	1430	1520	183
Lambda_z (hr-1)	0.238	0.353	0.297	0.298	0.0579
Term (hr)	1.96	2.92	2.40	2.32	0.482
PercAUCEstrap (%)	1.02	3.31	2.11	1.99	1.15
CL_F (L/hr)	8.51	12.0	10.2	10.1	1.74
V_F (L)	24.1	50.4	36.0	33.7	13.3

Table S3. TBR in ovarian cancer patients

Dose	TBR	Average tumor signal	Average background signal
0.0125mg/kg (N=3)	4.4	98.0	25.0
0.0125mg/kg extension (N=3)	5.4	68.0	14.4
0.025mg/kg (N=3)	4.1	112.9	29.5
0.05mg/kg (N=3)	3.5	112.8	33.9

Table S4. Listing of adverse events in the patient study

Listing of serious adverse events by patient

Subject	Treatment	Visit	Start Date/Time	Duration	Severity	SAE	Relationship	Action taken	Chronicity
8	OTL 0.0125 mg/kg	V1	21MAR2015 M	O	Mod	Yes	Unrelated	antibiotics	Single Occasion
			23MAR2015 M	O	Mod	Yes	Unrelated	surgical repair	Single Occasion

Listing of post dose adverse events by patient

Subject	Treatment	Visit	SOC [1]	Preferred Term	Signs and Symptoms	Start Date/Time	Duration	Time after (first) dosing	Severity	SAE	Relationship	Action taken	Chronicity	
2	OTL 0.025 mg/kg	V1	GASTR	Nausea	nausea	30SEP2014 M	O	M	Mild	No	Unrelated	ondansetron	Single Occasion	
					Vomiting	vomiting	30SEP2014 M	O	M	Mild	No	Unrelated	ondansetron	Single Occasion
				INJUR	Incision site swelling	pain and swelling wound groin	05OCT2014 M	O	M	Mild	No	Unrelated	Wait and see	Single Occasion
3	OTL 0.025 mg/kg	V1	GASTR	Nausea	nausea	27NOV2014 9:35	0D00H15M	0D00H05M	Mild	No	Related	no	Single Occasion	
					Nausea	nausea	27NOV2014 18:00	O	0D08H30M	Mild	No	Unrelated	no	Intermittent
				SKIN	Scar pain	wound pain	27NOV2014 18:00	O	0D08H30M	Mild	No	Unrelated	no	Single Occasion
4	OTL 0.05 mg/kg	V1	GASTR	Nausea	nausea	30DEC2014 9:55	0D00H15M	0D00H05M	Mild	No	Related	no	Single Occasion	
					Vomiting	vomiting	30DEC2014 9:55	0D00H05M	0D00H05M	Mod	No	Related	no	Single Occasion
					Abdominal discomfort	abdominal discomfort	30DEC2014 9:55	O	0D00H05M	Mild	No	Possible	no	Single Occasion
				GENRL	Fatigue	tired	31DEC2014 9:00	O	0D23H10M	Mild	No	Unrelated	no	Single Occasion
				RESP	Laryngeal discomfort	itch/ warm feeling throat	30DEC2014 9:55	0D00H15M	0D00H05M	Mild	No	Related	no	Single Occasion
					Nasal congestion	stuffed nose	30DEC2014 10:20	0D09H40M	0D00H30M	Mild	No	Probable	no	Single Occasion
	Sneezing	sneezing	30DEC2014 9:55	0D00H15M	0D00H05M	Mild	No	Related	no	Single Occasion				

Note: O=Ongoing after 2 weeks follow-up, M=missing

Listing of post dose adverse events by patient

Subject	Treatment	Visit	SOC [1]	Preferred Term	Signs and Symptoms	Start Date/Time	Duration	Time after (first) dosing	Severity	SAE	Relationship	Action taken	Chronicity
5	OTL 0.05 mg/kg	V1	EYE	Eye pruritus	itchy eyes	08JAN2015 10:35	0D00H15M	0D00H20M	Mild	No	Related	no	Single Occasion
			GENRL	Malaise	general malaise	09JAN2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
			RESP	Sneezing	sneezing	08JAN2015 10:24	0D00H01M	0D00H09M	Mild	No	Related	no	Single Occasion
			SKIN	Scar pain	wound pain	09JAN2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
		FU1	INFCT	Cystitis	cytitis	22JAN2015 7:00	O	NA	Mild	No	Unrelated	no	Single Occasion
6	OTL 0.05 mg/kg	V1	CARD	Tachycardia	tachycardia	10FEB2015 9:21	0D00H05M	0D00H03M	Mild	No	Probable	no	Single Occasion
			GASTR	Nausea	nausea	10FEB2015 9:21	0D00H12M	0D00H03M	Mild	No	Related	no	Single Occasion
				Vomiting	vomiting	10FEB2015 9:21	0D00H05M	0D00H03M	Mild	No	Related	no	Single Occasion
			GENRL	Fatigue	fatigue	11FEB2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
			SKIN	Scar pain	wound pain	11FEB2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
7	OTL 0.0125 mg/kg	V1	GASTR	Oral pruritus	burning sensation throat	05MAR2015 9:22	0D00H24M	0D00H12M	Mild	No	Related	no	Single Occasion
			GENRL	Fatigue	fatigue	06MAR2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
			SKIN	Eyelid irritation	heavy eyelids	05MAR2015 9:32	0D00H14M	0D00H22M	Mild	No	Probable	no	Single Occasion
Scar pain	wound pain	06MAR2015 M		O	M	Mild	No	Unrelated	no	Single Occasion			

Note: O=Ongoing after 2 weeks follow-up, M=missing

Listing of post dose adverse events by patient

Subject	Treatment	Visit	SOC [1]	Preferred Term	Signs and Symptoms	Start Date/Time	Duration	Time after (first) dosing	Severity	SAE	Relationship	Action taken	Chronicity
9	OTL 0.0125 mg/kg	V1	INJUR	Postoperative wound complication	wound problems	30MAR2015 M	O	M	Mild	No	Unrelated	conservative	Single Occasion
			SKIN	Intertrigo	intertrigo	06APR2015 M	O	M	Mild	No	Unrelated	conservative	Single Occasion
10	OTL 0.0125 mg/kg	V1	GASTR	Oropharyngeal pain	sore throat	30MAR2015 21:00	O	0D11H20M	Mild	No	Unrelated	no	Single Occasion
			GENRL	Fatigue	fatigue	01APR2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
11	OTL 0.0125 mg/kg	V1	GENRL	Fatigue	fatigue	09APR2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
			SKIN	Itching scar	skin irritation at scar	15APR2015 M	O	M	Mild	No	Unrelated	no	Single Occasion
12	OTL 0.0125 mg/kg	V1	GASTR	Abdominal discomfort	abdominal discomfort	04MAY2015 10:56	0D00H29M	0D00H09M	Mild	No	Related	no	Single Occasion
			SKIN	Scar pain	wound pain	05MAY2015 8:00	O	0D21H13M	Mild	No	Unrelated	no	Single Occasion

Note: O=Ongoing after 2 weeks follow-up, M=missing

Table S5. Number and characteristics of lesions and lymph nodes identified with fluorescence

Patient ID	Number of lesions identified with fluorescence			Number of lymph nodes identified with fluorescence		
	Total	True positive	False positive	Total	True positive	False positive
1	6	6	0	1	1	0
2	9	9	0	3	0	3
3	8	8	0	3	0	3
4	5	5	0	3	0	3
5	7	6	1	0	0	0
6	10	7	3	0	0	0
7	4	4	0	1	0	1
8	6	6	0	1	1	0
9	3	2	1	1	0	1
10	6	4	2	0	0	0
11	4	1	3	0	0	0
12	2	2	0	0	0	0
Total	70	60	10	13	2	11

Other supplementary files

Data File S1. Exploratory evaluation of potential hypersensitivity responses to compound OTL38 utilizing human, in vitro, highly sensitive models

Movie S1. Intraoperative fluorescence detection and resection of a lymph node containing ovarian cancer metastases