Abstract No. 435

Phase 1 trial of EO2463 peptide-based immunotherapy as monotherapy and in combination with lenalidomide and rituximab in indolent non-Hodgkin lymphoma: EONHL1-20/SIDNEY

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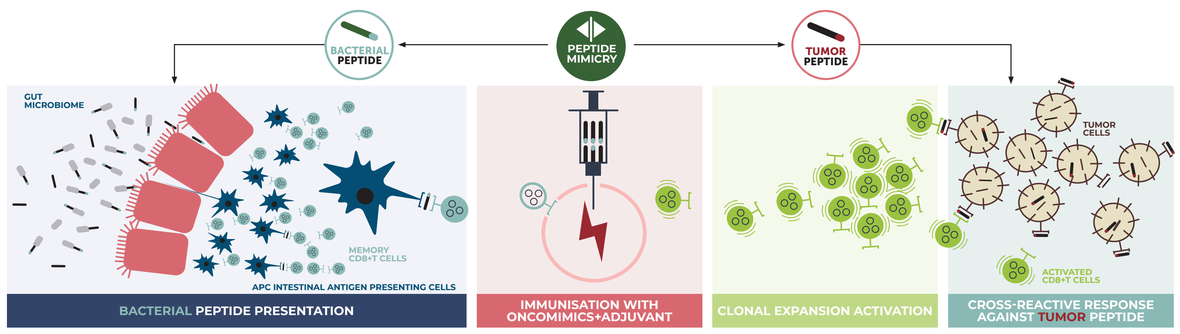
BACKGROUND

EO2463 was designed to expand pre-existing memory cytotoxic T cells recognizing specific protein sequences from gut bacteria with high prevalence in the population, which cross-react with B cell specific proteins to drive anti-tumor activity against B cell malignancies. EO2463 includes 4 synthetic peptides corresponding to CD8 HLA-A2 epitopes which exhibit molecular mimicry with the B cell antigens CD20, CD22, CD37, and CD268 (BAFF-receptor), as well as a helper CD4 peptide, UCP2, derived from hTERT. The EO2463 mimic peptides are non-self, high affinity and stable MHC class I binders.

The strategy aims at expanding cytotoxic T cells and is therefore expected to be less vulnerable to suppression of humoral immunity which is an effect of lenalidomide and/or rituximab also used in the study; however, to not interfere at all with early expansion of cytotoxic T cells, rituximab is delayed when utilized.

Memory CD8+ T cell clones can be detected in the peripheral blood of healthy donors recognizing the EO2463 mimic peptides; in vitro, such cells can kill target T2 cells loaded with mimic or human counterpart peptides.

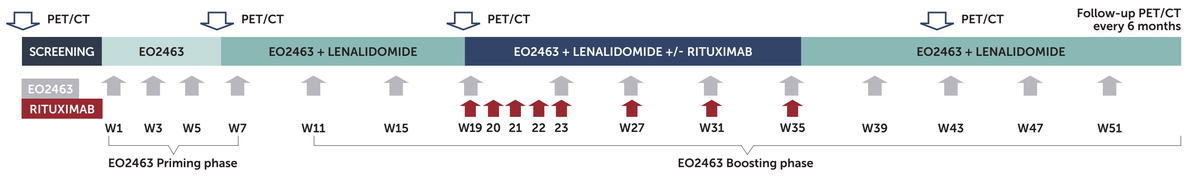


METHODS

This dose-finding safety lead-in phase 1 part of the ongoing phase 1/2 trial included patients with relapsed/refractory follicular lymphoma (FL) or marginal zone lymphoma (MZL) and investigated EO2463 + lenalidomide + rituximab in patients with Grade 1-3A FL or MZL, who received ≥ 1 line of prior systemic therapy. Patients are HLA-A2 with ECOG PS 0-2, no contraindications and measurable disease.

Patients received EO2463 q2 weeks x4, then every 4 weeks; administered SC with adjuvant Montanide ISA 51 VG up to 15 doses. After 6 weeks of EO2463 monotherapy, oral lenalidomide (20 mg/day for 21/28 days up to 12 cycles) was added, and if no complete remission (CR) at week 19, rituximab (375 mg/m^2 IV, weekly x 4, followed by q4 w infusions x 4) was also added. The peptide-dose was evaluated in a 3-by-3 safety design, starting at 150 µg/peptide, with a max escalation to 300 µg/peptide. Treatment was given until treatment completion, toxicity, or tumor progression.

The primary objective of the phase 1 part of the trial was to define the recommended phase 2 dose (RP2D) for EO2463 monotherapy, and to confirm the safety of EO2463 at the monotherapy RP2D in combination with lenalidomide (EL) and rituximab (ER^2).



SAFETY

EO2463 monotherapy

- No grade ≥3 related events occurred.
- Events attributed to EO2463 only, were local administration site reactions (LASRs) (erythema, inflammation and swelling) in 5/9 patients (3 Grade 2, and 2 Grade 1, events).
- The only other related event with EO2463 monotherapy was Grade 1 headache (1/9).
- EO2463 administration was delayed with one week for one patient due to LASR. No further dose modifications occurred.

EO2463 in combination with lenalidomide +/- rituximab

- The related events seen thus far were Grade 2 (1/9) and Grade 4 (2/9) neutropenia, Grade 3 anemia (1/9), Grade 2 leukopenia (1/9), Grade 3 thrombocytopenia (1/9), Grade 1 diarrhea (1/9), and Grade 3 rash (1/9).
- ER^2 was discontinued in one patient due to atrial fibrillation followed by heart failure and hematological toxicity (patient at the time in CR).

IMMUNE RESPONSE

Expansion of specific CD8+ T cells against the mimic peptides and targeted B cell antigens was detected in 6 of 8 tested patients, including in 2 patients (pat #3 and #4) with no measurable B cells at baseline (due to prior anti-CD20 therapy).

Expansion of specific T cells could be detected already after 2 administrations of EO2463 (week 5; note, week 3 not yet tested), and was maintained also following addition of rituximab.

The immune response against mimic peptides and B cell targets (cross-reactivity) could be detected directly ex vivo, using tetramer staining and flow cytometry on PBMCs.

In vitro expanded EO2463 specific CD8+ T cells from patients with immune response could kill malignant HLA-A2 B cell lines.

IMMUNE RESPONSE	PAT #1	PAT #2	PAT #3	PAT #4	PAT #5	PAT #6	PAT #7	PAT #8
Ex vivo (direct assay on PBMC) tetramer analyses percentage of specific CD8 T cells in peripheral blood								
Study week for testing	w 7	w35	w27	w19	w11	w7	w5	w7
Response to mimic peptides OMP64, OMP65, OMP66, and OMP72	No detection (only 2 peptides tested)	0.96%	5.18%	0.95%	No detection	0.47%	0.67%	0.98%
Response to B cell targets CD22, CD37, BAFF-R, and CD20	No detection (only 2 peptides tested)	0.82%	3.15%	0.93%	No detection	0.42%	0.49%	0.39%
IFN-γ ELISPOT (after 12 days in vitro stimulation) Spot forming cells/10^6 cells								
Study week for testing	w7	w 7	w7	w7	–	–	–	–
Peptide pool 4 mimic peptides	No expansion	8,420	10,307	2,980	Not done	Not done	Not done	Not done
Peptide pool 4 B cell targets	Not done	6,857	9,640	2,400	Not done	Not done	Not done	Not done

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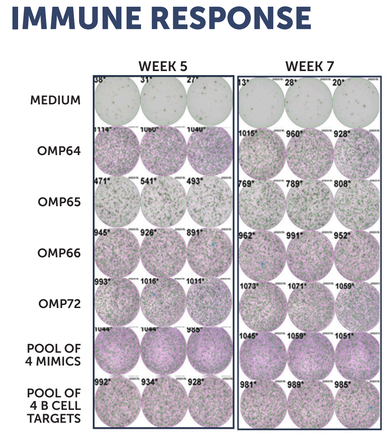
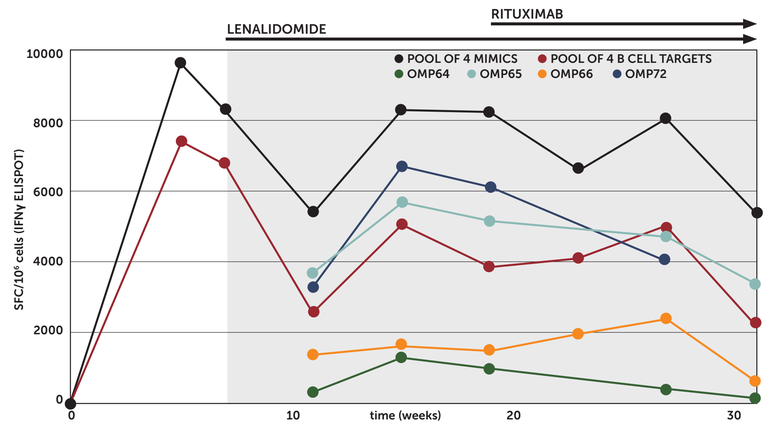
For open sites see clinicaltrials.gov NCT04669171.

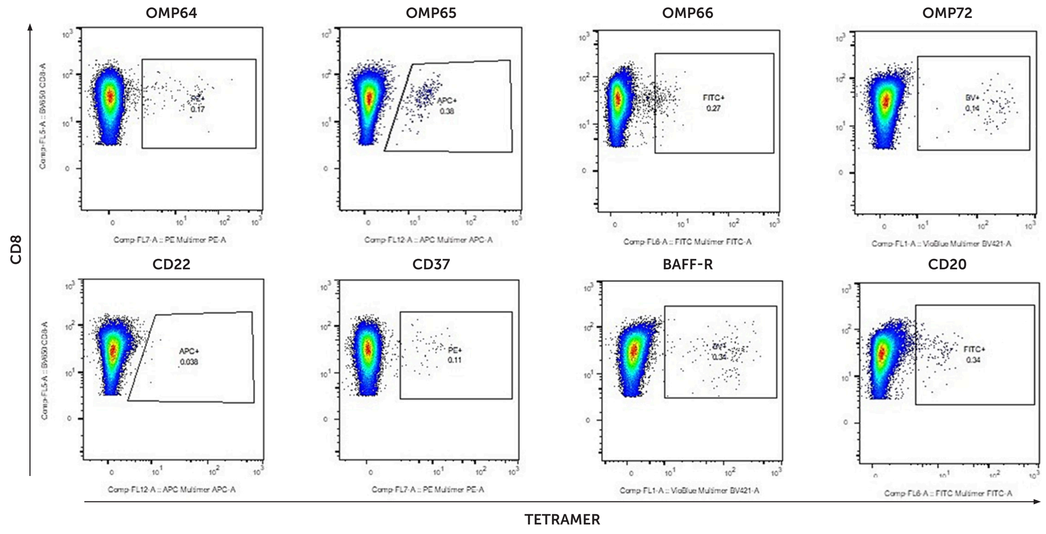
IMMUNE RESPONSE



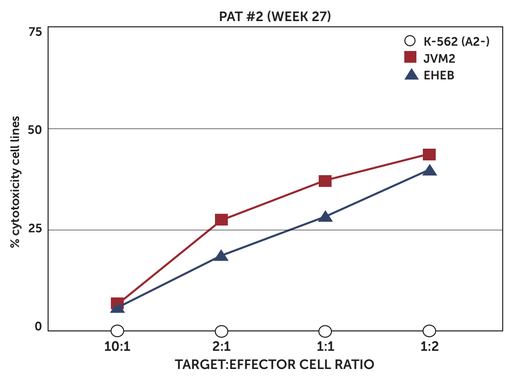
Pat #3: IFN-γ ELISPOT, 12 days in vitro stimulation with pool of 4 mimic peptides, restimulation with either individual mimic peptides, pool of mimics, or pool of B cell targets (cross-reactivity); 1 x 10^5 cells plated/well.

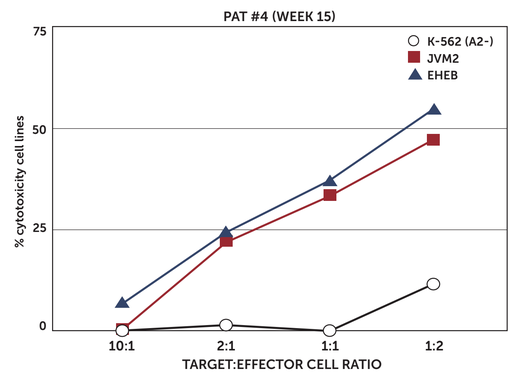


Pat #2: Immune response over time. Number of Spot forming cells by IFN-γ ELISPOT, 12 days in vitro stimulation with pool of 4 mimic peptides, restimulation with either individual mimic peptides, pool of mimics, or pool of B cell targets (cross-reactivity).



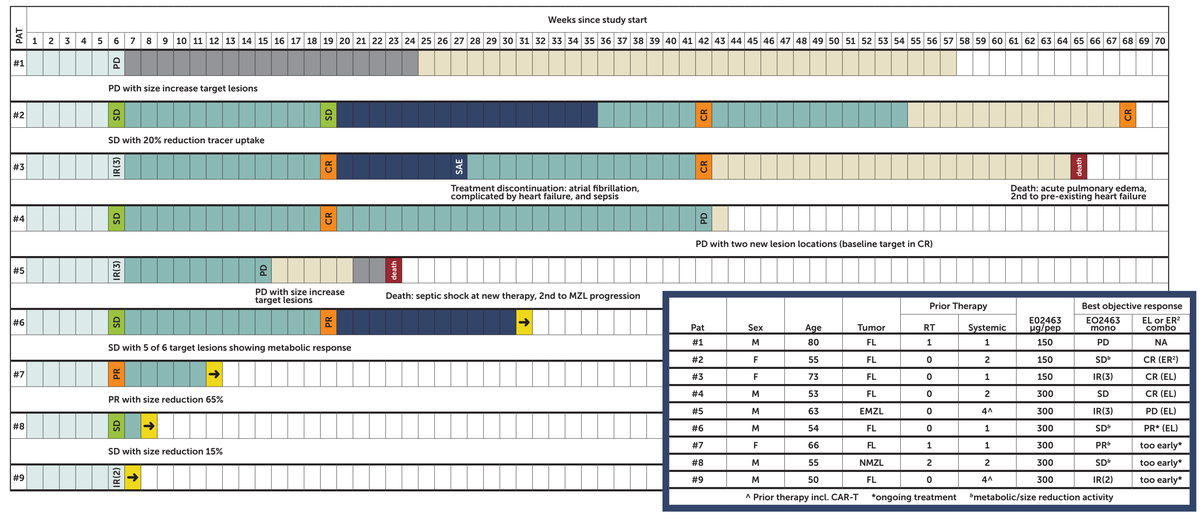
Pat #2 (week 35): Dot plots for ex vivo tetramer staining for mimic peptides (upper graphs) and B cell targets (lower graphs).





Pat #2 & #3: CD8 T cells were expanded from patient PBMCs ≥ 12 days by pool of 4 mimic peptides, and then assessed for cytotoxicity and cross-reactivity against two B cell target-expressing HLA-A2 lymphoblastic cell lines (JVM2 and EHEB) and an MHC class I deficient cell line (K562 cells) at different target/effector cells ratio. Killing was evaluated using cytometry and cell death marker.

CLINICAL OUTCOME



Pat	Sex	Age	Tumor	Prior Therapy: RT	Prior Therapy: Systemic	E02463 µg/pep	Best objective response: EO2463 mono	Best objective response: EL or ER^2 combo
#1	M	80	FL	1	1	150	PD	NA
#2	F	55	FL	0	2	150	SD[b]	CR (ER^2)
#3	F	73	FL	0	1	150	IR(3)	CR (EL)
#4	M	53	FL	0	2	300	SD	CR (EL)
#5	M	63	EMZL	0	4^	300	IR(3)	PD (EL)
#6	M	54	FL	0	1	300	SD[b]	PR* (EL)
#7	F	66	FL	1	1	300	PR[b]	too early*
#8	M	55	NMZL	2	2	300	SD[b]	too early*
#9	M	50	FL	0	4^	300	IR(2)	too early*

^ Prior therapy incl. CAR-T *ongoing treatment [b]metabolic/size reduction activity

CONCLUSIONS

- EO2463 is well tolerated as monotherapy, and without additional safety signals when combined with lenalidomide and rituximab.
- EO2463 generated fast, strong, and durable on-target immune activation with cytotoxic T cells with the ability to kill malignant HLA-A2 B cell lines in vitro.
- Clinical activity (metabolic/size reduction) in 4 of 9 (44%) patients on EO2463 monotherapy and encouraging preliminary complete response rate on combination therapy (50% in patients evaluable >week 19).
- The study phase 1 part, safety lead-in, findings justifies continuation of the trial (IDMC recommendation), including three expansion cohorts:
  - EO2463 monotherapy; patients with newly diagnosed, previously untreated, FL/MZL not in need of therapy (watch-and-wait setting)
  - EO2463 + rituximab (from w7-); patients with newly diagnosed, previously untreated, FL/MZL and low tumor burden, in need of therapy
  - EO2463 + lenalidomide (from w1-) + rituximab (from w19-); patients with relapsed/refractory FL/