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14. ABSTRACT

Small cell lung cancer (SCLC) has a dismal prognosis despite aggressive therapeutic approaches, and there is a clear need to develop more effective interventions. The role of tumor-initiating cells (TICs) in SCLC is largely unknown, although it is widely believed to be an important mechanism driving chemo-resistance in other cancers. Among cancers, SCLC is recognized for its rapid response to chemotherapy and equally rapid relapse. Thus, it is an ideal cancer in which to study TIC targeting, and drugs that selectively eradicate TICs offer great promise for treatment in this disease. Moreover, combinations of drugs will have more beneficial effects than a single agent.

Here we seek to develop an innovative and novel therapeutic regimen for SCLC by identifying synergistic combination therapies using two drugs, Rovalpituzumab tesirine (ROVA-T) and CBL0137 (CBL), both of which target SCLC TICs. ROVA-T (RT), a potent anti-cancer humanized antibody-drug conjugate, selectively targets delta-like protein 3 (DLL3), which is highly expressed in SCLC TICs. The experimental drug CBL has potent anticancer activity. CBL inhibits the histone chaperone Facilitates Chromatin Transcription (FACT), which is required for the expression of transcription factors that are essential for TIC maintenance. Thus, the TIC-targeting mechanisms of CBL and RT are entirely different, targeting two different proteins, FACT and DLL3 that are highly expressed in SCLC TICs and each is thought to control the tumor-initiating properties through different pathways. Furthermore, combination of TIC-targeting drugs with traditional chemotherapy may be especially effective in overcoming resistance. Chemotherapy preferentially targets non-TICs, which comprise the bulk of tumors, but spares self-renewing TICs, providing the rationale for our second approach; using CBL and RT together to synergize with the standard-of-care chemotherapeutic agent cisplatin, as a novel overall treatment strategy for SCLC. Our hope is that targeting both non-TICs and TICs simultaneously will eradicate the cancer more completely, reducing tumor burden and delaying or even preventing tumor recurrence.

15. SUBJECT TERMS
Small cell lung cancer, tumor-initiating cells, CBL0137, Rova-T, cisplatin

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INTRODUCTION:

Genomic profiling of SCLC is in its infancy, delaying the development of molecularly targeted therapies; so the most immediate therapeutic improvement against this cancer may depend on our ability to prevent or delay the emergence of chemo-resistance that accompanies traditional chemotherapy. We believe that targeting TICs will accomplish this goal. Therefore, our objective is to develop novel therapeutic approaches that counteract relapse by eradicating TICs. However, no drug is likely to be curative as a single agent and, therefore, we propose an innovative and novel therapeutic regimen for SCLC, by testing the potential synergistic combination of two TIC-targeting drugs, Rovalpituzumab tesirine (Rova-T) and CBL0137 (CBL). CBL and Rova-T target entirely different proteins, FACT and DLL3, both of which are highly expressed in SCLC TICs, predicting increased efficacy of the combination of these drugs towards TICs. Furthermore, combination of TIC-targeting drugs with traditional chemotherapy may be especially effective in overcoming resistance. Chemotherapy preferentially targets non-TICs, which comprise the bulk of tumors, but spares self-renewing TICs, providing the rationale for our second approach; using CBL and Rova-T together to synergize with the standard-of-care chemotherapeutic agent cisplatin, as a novel overall treatment strategy for SCLC. Our hope is that targeting both non-TICs and TICs simultaneously will eradicate the cancer more completely, reducing tumor burden and delaying or even preventing tumor recurrence. These studies will lead to novel therapeutic approaches in SCLC, and will provide key supporting data for rapid translation into the clinic.

1. KEYWORDS:

Small cell lung cancer, SCLC, tumor-initiating cells, TICs, CBL0137, Rova-T, FACT, DLL3, cisplatin, combination therapy, patient-derived xenografts, PDXs, cell survival, tumor growth, stem cell transcription factors.

2. ACCOMPLISHMENTS:

What were the major goals of the project? What was accomplished under these goals? The goals are listed in the Specific Aims and are followed by relevant accomplishments.

Specific Aim 1: To test the hypothesis that combining Rova-T with CBL0137 is synergistic in killing SCLC TICS.

Accomplishment:

We performed most of the experiments proposed to test our hypothesis in Aim 1 and recently published the work in the British Journal of Cancer as a brief communication entitled "CBL0137 increases the targeting efficacy of Rovalpituzumab tesirine against tumour-initiating cells in small cell lung cancer" (attached).

Specific Aim 2: To test the hypothesis that combining RT and CBL increases the therapeutic efficacy of Cisplatin (CP)

Major Task 1

Subtask 1: Determine the therapeutic effect of Cisplatin in combination with RovaT (RT) and CBL0137 (CBL) in the SCLC cell lines.

To determine the therapeutic effect of Cisplatin in combination with RT and CBL, we performed cell survival assays in the SCLC cell lines, H82 and H526. The cells were treated with RT or control IgG as single agents, or in combination of RT with CBL or cisplatin, or RT with CBL and cisplatin for 72 h. Cell survival was determined using the CyQUANT Direct assay, which measures proliferation as well as cytotoxicity. We show that the three-way combination of RT+CBL+cisplatin has increased the sensitivity of the cells compared to RT+CBL or RT+Cisplatin (Fig. 1 A, B).

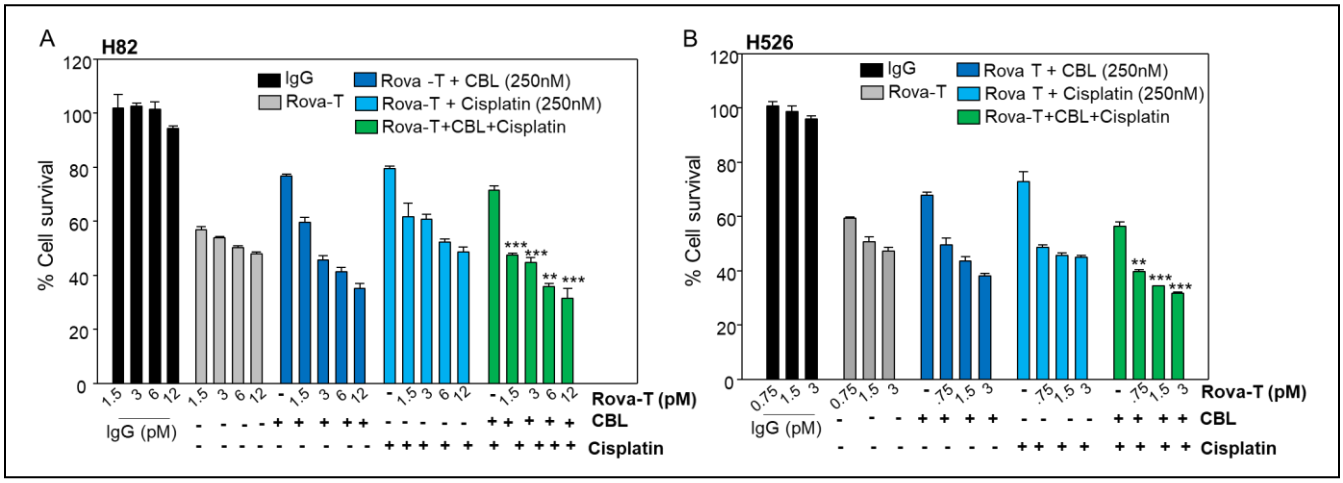

Fig. 1 SCLC cells are more sensitive to combination of Rova T (RT) + CBL0137 (CBL) + cisplatin than RT+CBL or RT+cisplatin. The cells were seeded at 3000 cells/well in black walled 96-well plates. Next day the cells were treated with IgG control or the drugs at different concentrations as shown in the figure. The cell viability after 72 h of treatment was determined and normalized to untreated controls. The experiments were repeated thrice and each measurement was performed in triplicate. Results are represented by means ± SD. Data were analyzed using Student's *t* test. *P* values of <0.05 are considered statistically significant. *, $P< 0.05$, **, $P< 0.01$, ***, $P< 0.001$, RT+CBL+cisplatin vs RT+CBL or RT+cisplatin.

Subtask 2: Determine the therapeutic efficacy of cisplatin in combination with RT and CBL in SCLC patient-derived xenograft (PDX)

In the pilot study, NOD/SCID mice were implanted with 50,000 PDX tumor cells and randomized into groups of 5 animals per cohort. Mice were treated with vehicle control for CBL + IgG control, cisplatin alone (5 mg/kg i.p., weekly), RT (1.8 mg/kg, i.p.,) + cisplatin, RT + CBL (60 mg/kg, i.v., once/ week), Cisplatin + CBL, or with combinations of RT with CBL and cisplatin. These studies suggested that Cisplatin + CBL+RT doses of 6/60/1.8 mg/kg were quite toxic.

Next, we performed the experiments by reducing the doses of cisplatin (4 mg/kg i.p., weekly) and CBL (50 mg/kg, i.v., once/ week). Even at reduced cis/cbl/rovaT doses of 4/50/1.8 mg/kg there was progressive weight loss that began shortly after RT administration (day 28) (Fig. 2 A). Toxicity seemed to be associated with addition of RT, since mice receiving cis alone or Ciplatin + CBL did not have weight loss (Fig. 2 B). All mice receiving RT in combinations were dead by day 44, whereas there was only 1/15 deaths in the other 3 groups. Tumor volumes (v) were calculated using the volume for a prolate spheroid: $v = 4/3 * \pi * a^2 * b$, where a = minor radius, b = major radius. Differences between

groups were analyzed by Student's t-test. There was no significant reduction in the tumor growth in the vehicle control vs cisplatin treated mice (P=0.745), vehicle control vs cisplatin + CBL treated mice (P= 0.706).

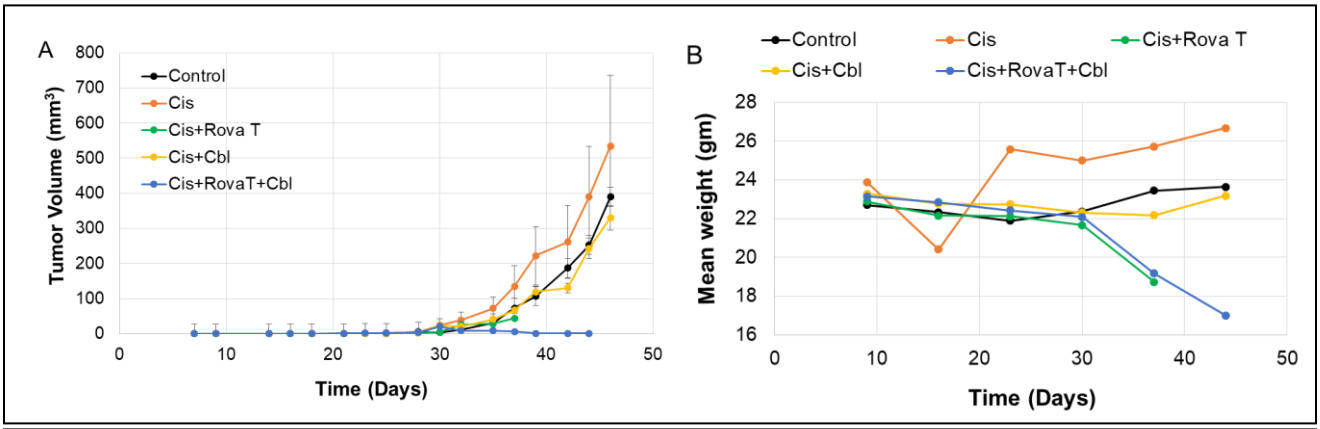

Fig.2. (A) Tumor volume in mice after treatment with combination of RT, cisplatin and CBL. SCLC PDX tumor cells were inoculated s.c. into the flanks of NSG mice. Once the tumors reached ~20 mm^3, the mice were randomized to treatment with vehicle + IgG control, Cisplatin (Cis), Cis + Rova T Cis + CBL0137 (CBL), Cis+CBL+RovaT. Tumors measured 3 times a week. The results are represented as mean ± SE. **(B)** The mean mouse body weight after treatment with control or drugs.

What opportunities for training and professional development has the project provided?
Nothing to report

How were the results disseminated to communities of interest?
Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to report

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Our findings together suggest that Rova T in combination with CBL0137 exerts a potent inhibitory effect on TICs derived from SCLC cell lines, significantly decreased tumor growth in SCLC PDXs, and increases the days of survival of mice. Therefore, our preclinical studies report a novel and highly translatable therapeutic strategy of dual targeting TICs using Rova-T in combination with CBL to potentially increase survival of SCLC patients.

What was the impact on other disciplines?
Nothing to report
What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. **CHANGES/PROBLEMS:** Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report

Changes that had a significant impact on expenditures
Nothing to report

6. **PRODUCTS:**
Nothing to Report

5. PARTICIPANTS & OTHER COLLABORATING ORGANIZATION

What individuals have worked on the project?

Name:	Sarmishtha De, PhD
Project role:	PI
Researcher identifier:	N/A
Nearest person months worked:	12
Contribution to project:	PI- Dr. De has worked oversight and direction.
Funding support:	This grant
Name:	Daniel Lindner, MD, PhD
Project role:	Collaborator
Researcher identifier:	N/A
Nearest person months worked:	6
Contribution to project:	All mouse experiments were performed in collaboration with Dr. Lindner.
Funding support:	This grant
Name:	Afshin Dowlati, MD
Project role:	Collaborator
Researcher identifier:	N/A
Nearest person months worked:	12
Contribution to project:	Dr. Dowlati has provided research advice, and reagents.
Funding support:	N/A
Name:	George Stark, PhD
Project role:	Collaborator
Researcher identifier:	N/A
Nearest person months worked:	12
Contribution to project:	Dr. stark has provided advice on all aspects of this research.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Nothing to report

8. SPECIAL REPORTING REQUIREMENTS
Nothing to report

9. APPENDIX:

A published manuscript that builds on this project is uploaded

Lindner DJ, Wildey G, Parker Y, Dowlati A, Stark GR, De S. CBL0137 increases the targeting efficacy of Rovalpituzumab tesirine against tumour-initiating cells in small cell lung cancer. Br J Cancer. 2020 Dec 1. doi: 10.1038/s41416-020-01192-x. Epub ahead of print. PMID: 33257843.

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BRIEF COMMUNICATION

Translational Therapeutics

CBL0137 increases the targeting efficacy of Rovalpituzumab tesirine against tumour-initiating cells in small cell lung cancer

Daniel J. Lindner[1], Gary Wildey[2], Yvonne Parker[1], Afshin Dowlati[2], George R. Stark[3] and Sarmishtha De[3]

Small cell lung cancer (SCLC) is characterised by high relapse rates. Tumour-initiating cells (TICs) are responsible for drug resistance and recurrence of cancer. Rovalpituzumab tesirine (Rova-T), a potent humanised antibody–drug conjugate, selectively targets delta-like protein 3, which is highly expressed in SCLC TICs. The experimental drug CBL0137 (CBL) inhibits the histone chaperone FACT (facilitates chromatin transcription), which is required for the expression of transcription factors that are essential for TIC maintenance. Rova-T and CBL each target SCLC TICs as single agents. However, acquired or intrinsic resistance to single agents is a major problem in cancer. Therefore, we investigated the potential effect of combining Rova-T and CBL in SCLC to eradicate TICs more effectively. Our preclinical studies report a novel and highly translatable therapeutic strategy of dual targeting TICs using Rova-T in combination with CBL to potentially increase survival of SCLC patients.

British Journal of Cancer https://doi.org/10.1038/s41416-020-01192-x

BACKGROUND

Small cell lung cancer (SCLC) has very high mortality because of its high relapse rate after standard-of-care therapies, coupled with a lack of effective second-line therapies. Tumour-initiating cells (TICs) within most solid tumours, including SCLC,[1] are important contributors to disease recurrence, metastasis and therapeutic resistance.[2,3] TICs can be identified by a high expression of specific marker proteins, such as CD133, compared with the bulk tumour cell population.[4]

Rovalpituzumab tesirine (Rova-T) is an antibody–drug conjugate (ADC) that comprises a humanised anti-delta-like protein 3 (DLL3) monoclonal antibody attached to a DNA-damaging pyrrolobenzodiazepine toxin.[5] Rova-T is considered to be the first biomarker-directed treatment for SCLC and is particularly effective against TICs.[5]

CBL0137 (CBL) targets FACT (facilitates chromatin transcription), a histone chaperone that is expressed at high levels in tumours and is required for the expression of transcription factors that are essential for TIC maintenance.[6,7] Recently, we reported that CBL as a single agent preferentially targets TICs in SCLC and has potent anti-cancer activity against SCLCs when combined with cisplatin.[8]

Thus, the TIC-targeting mechanisms of CBL and Rova-T are entirely different, targeting two different proteins, FACT and DLL3, respectively, that are highly expressed in SCLC TICs. Here, we investigated the therapeutic efficacy of these drugs in combination using both in vitro and patient-derived xenograft (PDX) models of SCLC. PDXs are well recognised as predictors of human cancer biology and patient response to treatment. The objective of these preclinical studies was to report a novel and highly translatable therapeutic strategy of dual targeting TICs that can potentially increase survival of SCLC patients.

METHODS

The $CD133^{high}$ (TIC) and $CD133^{low}$ (non-TIC) cells were generated from NCI-H82 (H82) and NCI-H526 (H526) as described previously.[8] The PDX tumour (JHU-LX102), derived from a chemotherapy-naive SCLC patient, was a generous gift from Dr. Charles M. Rudin (Memorial Sloan Kettering Cancer Center, New York). NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ mice, commonly known as the NOD *scid* IL-2 receptor gamma knockout (NSG) mice, were inoculated in both flanks with 50,000 tumour cells in each and randomised into cohorts of four animals per arm with mean starting tumour volumes of 100 mm^3. Mice were treated with vehicle control for CBL + IgG control, Rova-T alone, CBL alone or the combination of Rova-T with CBL.[5,8] Tumour volumes were measured three times a week until vehicle-treated mice reached ~1200 mm^3, at which time all mice were euthanised using a gradient controlled CO_2 inhalation, followed by cervical dislocation, and the tumours removed. In a second experiment, tumour-bearing mice were treated with the vehicle controls and the drugs as above, but now each group was treated until tumours reached the maximum size of ~1200 mm^3. For in vivo limiting dilution assays, cell suspensions from residual tumours harvested from the tumorigenicity studies were inoculated subcutaneously in limiting dilutions (10^3–10^5) into naive NSG mice.[9] The TIC frequency (TIF) was calculated using the ELDA software (http://bioinf.wehi.edu.au/software/elda/).[10]

RESULTS

Combination of Rova-T and CBL increases anti-tumour efficacy in vitro and in vivo by decreasing tumour-initiating frequency

Rova-T kills SCLC TICs by targeting DLL3.[5] We observed a higher expression of DLL3 in TICs of H82 and H526 compared to non-TICs

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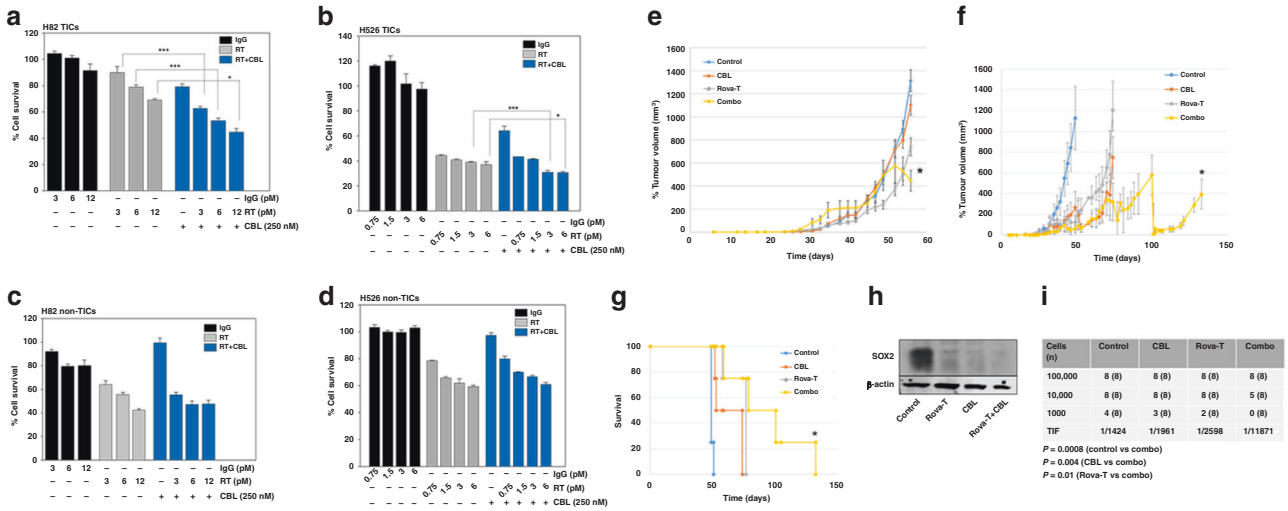

Fig. 1 Anti-tumour efficacy of combining Rova-T and CBL in vitro and in vivo. a H82 TICs, **b** H526 TICs, **c** H82 non-TICS and **d** H526 non-TICs were seeded at 3000 cells per well in black-walled 96-well plates. The next day cells were treated with IgG control or Rova-T at different concentrations, or CBL, or with Rova-T + CBL. The cell viability after 72 h of treatment was determined using the CyQUANT Fluorescent Assay[8] and normalised to controls. The experiments were repeated thrice, and each measurement was performed in triplicate. Results are represented by means ± SD. Data were analysed using Student's *t* test. *P* values of <0.05 are considered statistically significant. *$P < 0.05$, and ***$P < 0.001$. **e**, **f** Tumour volumes in mice after treatment with the combination of Rova-T and CBL. Four PDX mice with tumours in both the flanks ($N = 8$) were treated with vehicle controls for CBL + IgG control, Rova-T alone (1.8 mg/kg, i.p.), CBL alone (60 mg/kg, i.v., once per week) or combinations of Rova-T with CBL. Mice were treated with vehicle controls or CBL + IgG control, Rova-T alone, CBL alone or combinations of Rova-T with CBL. **e** Tumour volumes were measured in all groups until they reached ~1200 mm^3 in the vehicle-treated mice. **f** The treatment at the same doses as above was continued until the tumour volumes reached ~1200 mm^3 in each group. Tumour volumes (*v*) were calculated using the volume for a prolate spheroid: $v = 4/3 \times \pi \times a^2 \times b$, where *a* is the minor radius and *b* the major radius. Differences between groups were analysed by the Student's *t* test. The results are represented as means ± SE. * indicates $P < 0.05$ versus the single drug treatment groups. **g** Survival of mice shown in **f** ($P < 0.05$ for the combination vs single drugs alone). **h** SOX2 protein level was determined by Western analysis in the residual tumours derived from mice after treatment with vehicle for CBL + IgG control, or with CBL, Rova-T or Rova-T + CBL. β-Actin was used as a loading control. **i** In vivo limiting dilution assay showing that combining Rova-T and CBL reduced the tumour-initiating frequency (TIF). Mice were scored positive for tumour growth when the tumour size exceeded 200 mm^3 at 6 months after tumour cell inoculation. The TIF was calculated from $N = 8$ mice per group for each dilution of cells.

(Supplementary Fig. 1A–C), and treatment with CBL had no effect on DLL3 expression in TICs (Supplementary Fig. 1D), suggesting that CBL does not interfere with Rova-T efficacy by decreasing DLL3 levels. The combination of Rova-T and CBL decreased cell survival in H82 and H526 TICs much better than either drug alone (Fig. 1a, b). Importantly, the drug combination had no additional effect on the sensitivity of non-TICs compared to the single drugs alone (Fig. 1c, d), emphasising the preferential targeting of TICs by these drugs.

We compared the anti-tumour efficacy of Rova-T combined with CBL against single agents in a SCLC PDX model. There was no significant reduction in tumour growth in the mice treated with Rova-T plus CBL until day 50 compared to the groups treated with the individual drugs. However, tumour sizes started decreasing significantly ($P < 0.05$) after day 55 in the combination group (Fig. 1e). Since the vehicle-treated group had already reached the maximum allowed size by that day, we sacrificed the mice in all groups at that time. In a second experiment, tumour-bearing mice were treated with the vehicle controls or drugs at the same doses as above, but in this experiment, the mice in each group were treated until their tumours reached the maximum size of ~1200 mm^3. Treatment was started on day 31 after inoculation. Rova-T in combination with CBL substantially inhibited tumour growth, compared to Rova-T alone ($P < 0.05$), CBL alone ($P < 0.05$) or vehicle control ($P < 0.05$) (Fig. 1f). We observed that mice treated with combined Rova-T and CBL survived for 133 days, whereas vehicle-treated mice survived for 51 days, and mice treated with either CBL or Rova-T survived for 74–77 days (Fig. 1g). We could also show that the well-established cancer stem cell marker SOX2 level was decreased in the residual tumours from mice treated with CBL and Rova-T, compared to controls, with the drug combination being most effective (Fig. 1h). Rova-T treatment decreased DLL3 expression in residual tumours (Supplementary Fig. 2), indicating that Rova-T kills DLL3-expressing TICs. These results indicate that Rova-T in combination with CBL decreased the growth of SCLC PDX tumours and also increased survival by killing TICs, revealing a novel potent combination therapy for this cancer. We did not observe any toxicity in animals during treatment.

The limiting dilution assay is a rigorous test to quantitate cellular tumour-initiating capacity within a heterogeneous cancer cell population. Both Rova-T[5] and CBL[7,8] as single agents reduce tumour-initiating capacity in vivo[4] or in vitro.[7,8] To determine whether Rova-T combined with CBL can reduce tumour recurrence by targeting TICs, we performed in vivo limiting dilution assays on residual tumours. Tumours derived from control mice were shown to have a TIF of 1:1424, which was reduced to 1:1961 and 1:2598 in CBL or Rova-T-treated mice, respectively. A substantial reduction in the TIF to 1:11,871 was observed in tumours derived from mice treated with the combination of Rova-T and CBL (Fig. 1i).

DISCUSSION

SCLCs contain a much higher percentage of TICs than non-SCLCs, 65–75% compared to 15–20%,[11] and may therefore represent an ideal cancer in which to target TICs, which are relatively insensitive to chemotherapy and seed the growth of newly resistant tumours. While Rova-T and CBL have each been shown to target SCLC TICs as single agents,[5,8] it is unlikely that any drug will be curative as a single agent. Our findings show that the combination of two different TIC inhibitors, targeting two different proteins, DLL3 and

FACT, is much more toxic to SCLC TICs than either drug alone, and this therapeutic strategy is effective in vivo.

Tumour TICs can self-renew, differentiate and give rise to a new tumour. We reveal that the combination of Rova-T and CBL decreases SOX2 and attenuates the in vivo self-renewal capability of SCLC tumours by eradicating TICs, and thereby may also help to counteract relapse.

CBL is currently in the final stages of multicentre phase I clinical trials in advanced or metastatic solid tumours and lymphomas (NCT01905228), and it has not yet exhibited dose-limiting toxicity. Therefore, using CBL in combination with Rova-T may add therapeutic value to disappointing recent results with Rova-T alone in SCLC,[12,13] and could represent a novel drug combination that can prevent tumour recurrence and yield a more durable response in this cancer.

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AUTHOR CONTRIBUTIONS

D.J.L. and Y.P. performed the in vivo experiments and S.D. the in vitro experiments. D. J.L., G.W., A.D, G.R.S. and S.D. were involved in designing the study, obtaining research materials and editing the paper. S.D. supervised the study and wrote the initial manuscript.

ADDITIONAL INFORMATION

Ethics approval and consent to participate All animal experiments were approved by the Cleveland Clinic Foundation Institutional Animal Care and Use Committee and conducted in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (Protocol Number: 2017-1863). The cell lines used in this study were obtained from ATCC.

Consent to publish Not applicable.

Data availability All data generated or analysed during this study are included in this article and its supplementary information files.

Competing interests The authors declare no competing interests.

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Supplementary information is available for this paper at https://doi.org/10.1038/s41416-020-01192-x.

REFERENCES

1. Codony-Servat, J., Verlicchi, A. & Rosell, R. Cancer stem cells in small cell lung cancer. *Transl. Lung Cancer Res.* **5**, 16–25 (2016).
2. Wang, J., Li, Z. H., White, J. & Zhang, L. B. Lung cancer stem cells and implications for future therapeutics. *Cell Biochem. Biophys.* **69**, 389–398 (2014).
3. Eramo, A., Haas, T. L. & De Maria, R. Lung cancer stem cells: tools and targets to fight lung cancer. *Oncogene* **29**, 4625–4635 (2010).
4. MacDonagh, L., Gray, S. G., Breen, E., Cuffe, S., Finn, S. P., O'Byrne, K. J. et al. Lung cancer stem cells: the root of resistance. *Cancer Lett.* **372**, 147–156 (2016).
5. Saunders, L. R., Bankovich, A. J., Anderson, W. C., Aujay, M. A., Bheddah, S., Black, K. et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. *Sci. Transl. Med.* **7**, 302ra136 (2015).
6. Gasparian, A. V., Burkhart, C. A., Purmal, A. A., Brodsky, L., Pal, M., Saranadasa, M. et al. Curaxins: anticancer compounds that simultaneously suppress NF-kappaB and activate p53 by targeting FACT. *Sci. Transl. Med.* **3**, 95ra74 (2011).
7. Dermawan, J. K., Hitomi, M., Silver, D. J., Wu, Q., Sandlesh, P., Sloan, A. E. et al. Pharmacological targeting of the histone chaperone complex FACT preferentially eliminates glioblastoma stem cells and prolongs survival in preclinical models. *Cancer Res.* **76**, 2432–2442 (2016).
8. De, S., Lindner, D. J., Coleman, C., Wildey, G., Dowlati, A., Stark, G. R. et al. The FACT inhibitor CBL0137 synergizes with cisplatin in small cell lung cancer by increasing NOTCH1 expression and targeting tumor-initiating cells. *Cancer Res.* **78**, 2396–2406 (2018).
9. Kolev, V. N., Wright, Q. G., Vidal, C. M., Ring, J. E., Shapiro, I. M., Ricono, Jill, D. et al. PI3K/mTOR dual inhibitor VS-5584 preferentially targets cancer stem cells. *Cancer Res.* **75**, 446–455 (2015).
10. Hu, Y. & Smyth, G. K. ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. *J. Immunol. Methods* **347**, 70–78 (2009).
11. Sullivan, J. P., Spinola, M., Dodge, M., Raso, M. G., Behrens, C., Gao, B. et al. Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. *Cancer Res.* **70**, 9937–9948 (2010).
12. Van Den Borg, R., Leonett, A., Tiseo, M., Giovannetti, E. & Peters, G. J. Novel targeted strategies to overcome resistance in small-cell lung cancer: focus on PARP inhibitors and rovalpituzumab tesirine. *Expert Rev. Anticancer Ther.* **19**, 461–471 (2019).
13. Morgensztern, D., Besse, B., Greillier, L., Davila, R. S., Ready, N., Christine, L. et al. Efficacy and safety of rovalpituzumab tesirine in third-line and beyond patients with DLL3-expressing, relapsed/refractory small-cell lung cancer: results from the Phase II TRINITY Study. *Clin. Cancer Res.* **25**, 6958–6966 (2019).