Intracavitary 'T4 immunotherapy' of malignant mesothelioma using pan-ErbB re-targeted CAR T-cells

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ABSTRACT

Malignant mesothelioma remains an incurable cancer. We demonstrated that mesotheliomas expressed EGFR (79.2%), ErbB4 (49.0%) and HER2 (6.3%), but lacked ErbB3. At least one ErbB family member was expressed in 88% of tumors. To exploit ErbB dysregulation in this disease, patient T-cells were engineered by retroviral transduction to express a panErbB-targeted chimeric antigen receptor (CAR), co-expressed with a chimeric cytokine receptor that allows interleukin (IL)-4 mediated CAR T-cell proliferation. This combination is referred to as T4 immunotherapy. T-cells from mesothelioma patients were uniformly amenable to T4 genetic modification and expansion/ enrichment thereafter using IL-4. Patient-derived $T4^+$ T-cells were activated upon contact with a panel of four mesothelioma cell lines, leading to cytotoxicity and cytokine release in all cases. Adoptive transfer of T4 immunotherapy to SCID Beige mice with an established bioluminescent LO68 mesothelioma xenograft was followed by regression or eradication of disease in all animals. Despite the established ability of T4 immunotherapy to elicit cytokine release syndrome in SCID Beige mice, therapy was very well tolerated. These findings provide a strong rationale for the clinical evaluation of intracavitary T4 immunotherapy to treat mesothelioma.

Key words

Lung cancer; mesothelioma; chimeric antigen receptor; epidermal growth factor receptor

Abbreviations

BLI – bioluminescence imaging; CAR – chimeric antigen receptor; EGFR – epidermal growth factor receptor; ffLuc – firefly luciferase; IL – interleukin; i.p. - intraperitoneal; MPM

– malignant pleural mesothelioma; PSMA – prostate-specific membrane antigen; p/s – photons per second; RFP – red fluorescence protein; UT - untransduced

1. Introduction

Malignant mesothelioma is a locally aggressive tumor that is primarily attributable to inhalation of asbestos fibers [1], prompting stringent regulation of this mineral in many territories. Nonetheless, incidence continues to increase in many countries, reflecting the long latency period of disease progression [2]. Mesothelioma remains incurable with a median survival of 9 to 17 months [3, 4].

Immunotherapy has long been posited as a logical approach to treat mesothelioma [5]. Promising data have emerged from clinical trials of immune checkpoint blockade in this disease [6]. In parallel, there has been increasing interest in the development of adoptive immunotherapeutic approaches using genetically engineered T-cells. Chimeric antigen receptors (CARs) are bespoke fusion molecules that couple an antigen recognition element to a tailored signaling domain, thereby enabling the HLA independent retargeting of T-cell specificity [7]. Interest has been fueled by the repeated demonstration of unprecedented therapeutic efficacy when autologous CD19 targeted CAR T-cells are administered to patients with refractory acute lymphoblastic leukemia [8]. The most successful CAR configurations employ a second generation design in which CD28 or 4-1BB costimulatory modules are fused to CD3ζ [9-11]. While initial experience in patients with solid tumors has been disappointing, mesothelioma may represent a special case since tumors are amenable to regional CAR T-cell delivery to the affected body cavity. Preclinical data have provided strong support for this approach using CAR T-cells retargeted against mesothelin [12, 13]

and fibroblast activation protein [14, 15]. Clinical trials employing both intra-pleural [16, 17] and systemic delivery [18] are ongoing.

An increasing concern with CAR T-cell immunotherapy is the emergence of therapeutic resistance due to target loss [19, 20]. This highlights the desirability of selecting multiple targets that contribute to disease pathogenesis and thus are subject to selective pressure for retention by tumors. In mesothelioma, the extended ErbB receptor family represents an attractive candidate to meet these requirements. Epidermal growth factor receptor (EGFR; ErbB1) is upregulated and phosphorylated in the majority of cases, compared to normal pleura [21-27]. Disappointingly however, clinical evaluation of EGFR tyrosine kinase inhibitors has proven ineffective in this disease [24, 25], while trials involving EGFR inhibitory antibodies are ongoing (www.clinicaltrials.gov, accessed February 5th, 2017). Co-expression of HER2 (ErbB2) [24, 28] has also been reported, although ErbB3 and ErbB4 have not previously been evaluated.

To redirect T-cell specificity against the ErbB family, we have engineered a second generation CAR named T1E28z. T1E28z engages 8 of 9 possible ErbB homo and heterodimers [29] and enables T-cells to elicit antitumor activity in preclinical models of ovarian, breast and head and neck cancers [29-31]. The CAR is co-expressed with a chimeric cytokine receptor named 4αβ that delivers an interleukin (IL)-2/IL-15 signal upon binding of IL-4, thereby enabling the selective enrichment of CAR T-cells during *ex vivo* expansion [32]. Importantly, culture of the resulting '$T4^{+}$' T-cells in IL-4 does not alter their differentiation and maintains type 1 polarity [32]. T4 immunotherapy is currently undergoing Phase 1 evaluation by intra-tumoral delivery in patients with head and neck cancer [33]. This approach has a further theoretical advantage in mesothelioma treatment since IL-4 is commonly found in the tumor microenvironment, providing an additional source of stimulation for $T4^{+}$ T-cells [34]. Here, we tested the hypothesis that $T4^{+}$ T-cells derived from

patients with mesothelioma would exert meaningful antitumor activity against mesothelioma, justifying the clinical evaluation of this approach.

2. Materials and methods

Additional details are provided as an online supplement.

2.1 Preparation of tissue microarray slides and immunohistochemistry of tumors

Mesothelioma tissue microarrays (TMAs) were acquired from the Departmental Tissue Bank. Sections (3μm) were cut from formalin fixed paraffin-embedded TMA blocks of 168 mesotheliomas obtained at a surgical biopsy procedure (either video-associated thoracoscopic surgery or open pleural biopsy), and were prepared on appropriately coated slides.

Access to patient tissue samples and data is regulated under the Human Tissue Act and Guy's and St Thomas' Hospital Thoracic Cancer Biobank Access Committee (License number 12121) in accordance with NHS Research Ethics Committee conditions.

After overnight incubation, the slides were dewaxed and endogenous peroxidase was blocked by treatment with 3% hydrogen peroxide for 30 min. Antigen was retrieved by microwaving slides in citrate buffer pH 6.0 for 3 x 4 minutes. Primary antibodies (all Abcam, Cambridge, UK) were rabbit polyclonal anti-EGFR (ab2430; 1:100), rabbit monoclonal anti-ErbB2 (ab134182; 1:200), rabbit monoclonal anti-ErbB3 (ab93739; 1:200) and mouse monoclonal anti-ErbB4 (ab19391; 1:20). Each was applied for 60 min in humidified chambers at room temperature, and staining was performed with the avidin-biotin complex by using the VECTASTAIN ABC Elite kit (PK-6200, Vector Laboratories, CA). 3,3-Diaminobenzidine (DAB) was used as a chromogen (Vector Laboratories, CA). Slides were

lightly counterstained with hematoxylin. Breast carcinoma sections were used as positive and negative controls for EGFR, HER2 and ErbB3. A pellet of A431 cells was used as positive control for ErbB4. For all negative controls, normal horse serum was used instead of primary antibody. Following staining, TMA slides were scored for intensity of staining by an independent histopathologist, who reported results as negative (absence of staining), low intensity (+), moderate intensity (++) or high intensity (+++).

2.2 Genetic modification of patient T-cells

This study was approved by the Leeds West Research Ethics Committee (reference 12/YH/0392). Recombinant DNA constructs were expressed using the SFG retroviral vector. Retroviral transduction [35] and expansion of CAR T-cells using IL-4 was carried out as described [32].

2.3 Cytotoxicity and cytokine release assays

Monolayers of mesothelioma cell lines were incubated with $1x10^6$ T-cells overnight, (effector:target ratio of 1:1). Cytokine measurements were performed by ELISA. Tumor cell viability was assessed by MTT assay.

2.4 Animal studies

Experimentation complied with UK Home Office project license 70/7794. SCID Beige mice were inoculated by intraperitoneal (i.p.) injection with $2x10^6$ retrovirus-transduced LO68 tumor cells that co-express firefly luciferase (ffLuc) and red fluorescent protein (RFP) and were flow sorted to homogeneity. Tumor engraftment was confirmed by bioluminescence imaging (BLI) using the IVIS Spectrum Imaging platform and Living Image software (PerkinElmer, Waltham, MA, USA). Mice were sorted into groups based on

similar mean total flux. Patient-derived CAR T-cells were administered i.p. and tumor status monitored by BLI [36]. Mice were culled if symptomatic, or when experimental endpoints had been met. For statistical comparison of two groups, datasets were analyzed by Student's *t* test.

3. RESULTS

3.1 Expression of ErbB receptors by malignant pleural mesotheliomas

Tissue microarrays were prepared from 168 malignant pleural mesotheliomas (MPM). Histologic subtype was defined in 103 cases as epithelioid (56.7%), sarcomatoid (20.4%) or biphasic (22.8%). As expected [37], survival was significantly longer for patients with epithelioid mesotheliomas, compared to other subtypes (Fig. 1A).

Immunohistochemical analysis (Table I and Fig. 1B) demonstrated that most tumors expressed EGFR, while ErbB4 was detected in half of cases. By contrast, HER2 expression was infrequent and ErbB3 was uniformly absent. At least one ErbB family member was expressed in 88% of tumors. ErbB receptor expression was strongly dependent upon histologic subtype, with highest expression in epithelioid tumors (Table I). Receptor distribution was mainly cytoplasmic (Supplementary Fig. S1).

Univariate analysis indicated that median patient survival was significantly influenced by EGFR expression status, but not by that of other ErbB family members (Fig. 1C). Survival was shortest when tumors either lacked EGFR, or were strongly positive for EGFR expression (Fig. 1C and Table II). Similar findings were observed when analysis was restricted to tumors for which histologic subtype data were available (Table II and Fig. 1D). When restricted to epithelioid tumors, effect of EGFR expression on survival was no longer statistically significant (Supplementary Fig. 2). Nonetheless, similar trends towards lower

median survival were apparent when tumors either lacked EGFR or expressed EGFR at high levels (Table II).

Multivariate analysis was undertaken to explore the interactive effects of EGFR and histologic status on patient survival. This demonstrated that histology remained a highly significant predictor of patient outcome. By contrast EGFR status was not a significant covariate, unless the analysis was restricted to the small number of tumors in which the highest level of EGFR positive was detected (Table III).

3.2 CAR engineering of T-cells from mesothelioma patients

Given the prevalent expression of ErbB receptors in mesotheliomas, we explored feasibility of generation of $T4^+$ T-cells using peripheral blood from mesothelioma patients. All were male, aged 69.9±7.3 years (mean ± SD) and had tumors of epithelioid (n=8), biphasic (n=2) or unspecified subtypes (n=2), at clinical stage T2N0 (n=6), T3N0 (n=5) or T4N2 (n=1). Blood was obtained prior to diagnostic surgery (n=10) or following chemotherapy (n=2). Control T-cells were transduced with SFG P4, which encodes for 4αβ and P28z [35]. P28z binds to PSMA, which is not expressed in mesothelioma.

Transduced CAR T-cells were selectively enriched by expansion in IL-4. Fig. 2 demonstrates that gene transfer and IL-4 mediated *ex vivo* enrichment of CAR T-cells was achieved in all cultures (representative example, Fig. 2A; pooled data, Fig. 2B-D). Total cell number increased by 28.1±13.9 fold, accompanied by IL-4 mediated enrichment of $T4^+$ T-cells by 4.7±4.8 fold (mean ± SD).

3.3 In vitro assessment of antitumor activity of ErbB retargeted T-cells

Co-cultivation assays were performed in which patient derived CAR T-cells were incubated with a panel of mesothelioma cell lines (REN, JU77, LO68 and H28). Tumor cell

lines expressed EGFR (Fig. 3A), HER2 (Fig. 3B) and ErbB4 (Fig. 3D), while low level expression of ErbB3 was detected in two cases (Fig. 3C). All four mesothelioma cell lines were destroyed by $T4^+$, but not by control $P4^+$ or untransduced T-cells (Fig. 4). Activation of $T4^+$ T-cells was indicated by release of significantly elevated amounts of IL-2 (Fig. 5A) and IFN-γ (Fig. 5B).

3.4 In vivo assessment of patient derived ErbB retargeted CAR T-cells – single dose study

An i.p. xenograft model was established in SCID Beige mice using ffLuc/RFP-expressing LO68 cells. This approach was selected for three reasons. First, tumor progression models the propensity of mesothelioma to undergo intracavitary spread at a location in which human mesotheliomas also occur. Second, this model allowed us to examine the therapeutic activity and safety of patient derived $T4^+$ T-cells, when delivered using a route that can cause cytokine release syndrome (CRS) [31]. Finally, i.p. delivery of tumor and T-cells is less traumatic to animals than intrapleural delivery.

Inoculation of either 0.5 or 2 million $ffLuc^+$ LO68 tumor cells led to engraftment followed by exponential tumor growth after 14 days (Supplementary Fig. S3). The higher tumor cell dose was selected for further studies. To examine *in vivo* antitumor activity, 40mL blood was obtained from a patient with epithelioid MPM (patient 31). T-cells were transduced with SFG T4 or the control SFG P4 vector and were expanded and enriched by culture in IL-4. The selective ability of this batch of $T4^+$ CAR T-cells to kill LO68 tumor cells was confirmed (Supplementary Fig. S4A) prior to their adoptive transfer to animals bearing a 14 day established LO68 tumor xenograft. Fig. 6A demonstrates that $T4^+$ patient T-cells cause a transient regression of tumor burden. By contrast, $P4^+$ T-cells exerted no therapeutic effect when compared to PBS. Treatment was extremely well tolerated with no

evidence of weight loss or other "soft" clinical indicators of CRS, such as ruffled fur, hunched posture or reduced mobility or grooming (Fig. 6B).

3.5 Repeated administration of patient-derived ErbB re-targeted CAR T-cells exerts superior therapeutic activity

We have recently shown that CAR T-cells are rapidly cleared from SCID Beige mice following i.p. delivery [38]. This contrasts to NSG mice where sustained engraftment leads to xenogeneic graft versus host disease (unpublished data). Consequently, we next explored the therapeutic efficacy and safety of repeated CAR T-cell dosing in the SCID Beige model. A 40mL blood sample was obtained from a patient with epithelioid MPM (patient 36) and CAR T-cells were engineered and expanded as above. Antitumor activity of $T4^+$ but not control T-cells was confirmed using LO68 tumor cells (Supplementary Fig. 4B). CAR T-cells were adoptively transferred into tumor-bearing mice on days 14 and 21. Fig. 7A-B demonstrates that tumor regression was achieved in all $T4^+$ T-cell treated mice, with disease eradication in 2 of 5 cases. No weight loss was observed in any mouse (Fig. 7C).

4. Discussion

Solid tumors impose considerable obstacles to effective CAR T-cell immunotherapy. The first hurdle is the delivery of sufficient numbers of T-cells to the site of disease. While disseminated metastasis represents the main therapeutic challenge imposed by most cancers, some tumors primarily advance by loco-regional dissemination. This scenario provides a unique opportunity for CAR T-cell immunotherapy since direct administration of T-cells to such lesions obviates their homing requirement from the bloodstream. Furthermore, pre-clinical studies demonstrate that regional CAR T-cell administration has a highly favorable

safety profile [31], with limited systemic absorption [39]. We have exploited this principle to initiate a clinical trial in which ErbB retargeted CAR T-cells are administered using the intra-tumoral route in patients with head and neck cancer [33]. To date, no dose limiting toxicities or evidence of systemic absorption of CAR T-cells has been observed in the first ten treated patients. Here, we demonstrate preclinical proof of concept for the utility of a related approach to treat mesothelioma. Mesothelioma is highly suited to intra-cavitary delivery since tumors are directly accessible using a pleural or peritoneal catheter [12, 16, 40].

We first profiled ErbB receptor expression in a large tumor series. The majority of MPM expressed EGFR, in agreement with previous reports [21-27]. Expression of EGFR has previously been linked to no alteration [21, 23, 25] or to improved survival of mesothelioma patients on univariate analysis [22, 27]. In agreement, we found that lack of EGFR was associated with shortened patient survival. Since this effect lost significance when the analysis was restricted to epithelioid tumors, it may in part reflect the tendency of poorer prognosis mesothelioma subtypes (notably sarcomatoid tumors) [41] to lack EGFR expression. Notably however, we also observed that in EGFR positive tumors, highest level expression was also linked to shortened survival. Although not been described previously, a similar trend was seen in a smaller study in which intensity of EGFR expression by mesotheliomas was ranked [21].

Expression of HER2 has previously been examined in mesothelioma, with divergent findings. Our finding that HER2 is infrequently detectable using immunohistochemistry is consistent with Garland *et al*. [24], but not with an earlier report in which 97% of tumors were positive [28]. In interpreting these findings, differing sensitivities of distinct immunohistochemical approaches should be considered.

We also undertook an evaluation of ErbB3 and ErbB4 expression by MPM, building on earlier studies using immortalized cell lines [42-44]. While ErbB3 was not detected, about

half of tumors expressed ErbB4. Once again, the limited sensitivity of immunohistochemistry should be considered in interpreting these findings. Nonetheless, these data indicate that several ErbB dimer species recognized by the T1E28z CAR are present in most mesotheliomas, notably epithelioid tumors.

Immunotherapy using CAR T-cells generally involves the use of an autologous product. Consequently, it was imperative to demonstrate feasibility of transduction and expansion of patient-derived CAR T-cell products. To facilitate this, T1E28z was co-expressed with the 4αβ chimeric cytokine receptor which allows selective enrichment of CAR T-cells by culture in IL-4 [32]. This approach yields up to 8 billion CAR T-cells within 2 weeks from 120mL blood, obtained from patients with advanced head and neck cancer (unpublished data). We show here that successful transduction and enrichment of CAR T-cells was achieved in all patients, either at diagnosis or following chemotherapy. Functionality of the expanded cells was indicated by their ability to kill four mesothelioma cell lines, with release of IL-2 and IFN-γ at significantly higher levels that control CAR T-cells (due to density of plating and alloreactivity). Furthermore, we obtained sufficient CAR T-cells from 40mL patient blood to treat 10 mice bearing an established mesothelioma xenograft. A single cycle of treatment with $T4^+$, but not control CAR T-cells led to transient tumor regression in all mice. Tumor response could be consolidated by the administration of a second CAR T-cell dose, causing disease eradication in 40% of animals. Notably, we have previously demonstrated that i.p. administration of $T4^+$ T-cells to SCID Beige mice causes dose-dependent CRS. This results from the ability of human CAR T-cells to activate mouse macrophages, leading to IL-6 release [31]. Reassuringly however, no such toxicity was evident at the doses of CAR T-cells used here.

In conclusion, we have demonstrated ErbB receptor expression in most mesotheliomas, particularly of the epithelioid subtype. Exploiting this, human ErbB-

retargeted CAR T-cells exert meaningful antitumor activity against mesothelioma cell lines and xenografts, without toxicity. These data provide strong support for the clinical evaluation of intracavitary T4 immunotherapy in patients with mesothelioma.

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6. Appendix A. Supplementary data

Supplementary data related to this article can be found at http…

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8. Figure legends

Fig. 1 Histology, ErbB receptor expression and survival analysis of patients with malignant pleural mesothelioma. (A) Kaplan Meier plot of survival of patients with mesotheliomas of the specified histologic subtypes. (B) Frequency and level of expression of indicated ErbB family members in tissue microarrays derived from 168 mesotheliomas. All tumors were negative for ErbB3. (C) Kaplan Meier plots of survival of patients with mesothelioma according to frequency and level of expression of ErbB family members. Data shown in B and C includes all tumors in which histologic subtype was either defined or not specified. (D) Re-analysis of plots shown in C but restricted to patients with mesothelioma of defined histologic subtype. *p* values were generated by Log-rank (Mantel Cox) test.

Fig. 2 Transduction efficiency and *in vitro* expansion of CAR T-cells from patients with malignant pleural mesothelioma. (A) Representative example in which patient-derived peripheral blood T-cells were transduced with the SFG T4 retroviral vector, which encodes for the ErbB-specific T1E28z CAR and 4αβ chimeric cytokine receptor. After gene transfer, IL-4 was provided as sole cytokine support during the expansion phase. Pooled data indicate IL-4-mediated enrichment of $T4^+$ CAR T-cells within the culture (B), numerical expansion of T-cells (C) or of $T4^+$ T-cells (D). Data are derived from 12 separate patients comparing day 3 and day 15 after transduction.

Fig. 3 ErbB receptor expression by malignant pleural mesothelioma cell lines. The indicated cell lines were analyzed by flow cytometry for (A) EGFr (ErbB1); (B) HER2 (ErbB2); (C)

ErbB3 and (D) ErbB4, making comparison with an isotype control for monoclonal antibody staining (A-C) or conjugated secondary antibody alone for polyclonal antiserum (D).

Fig. 4 *In vitro* cytotoxic activity of patient-derived ErbB re-targeted CAR T-cells against malignant pleural mesothelioma cell lines. CAR^+ (T4 or control P4) or untransduced (UT) T-cells were generated from 12 mesothelioma patients and were established in co-culture with the indicated tumor cell lines at a 1:1 ratio overnight. After removal of T-cells, residual viable tumor was quantified by MTT assay, making comparison with a parallel culture of tumor cells alone ("tumor" – set to 100%). Data show mean $\pm$ SD of n=12 assays from individual donors. **** $p<0.0001$ comparing T4 with all other conditions by Tukey's multiple comparisons test.

Fig. 5 Cytokine release by patient-derived ErbB re-targeted CAR T-cells when co-cultivated with malignant pleural mesothelioma cell lines. Co-cultures were established between CAR^+ (T4 or control P4) or untransduced (Untrans.) T-cells and the indicated tumor cell lines at a 1:1 ratio overnight. Supernatants were collected and analyzed by ELISA for IL-2 (A) or IFN-γ (B). Data show mean $\pm$ SD of triplicate samples from each patient-derived T-cell culture. ND – not done.

Fig. 6 *In vivo* anti-tumor activity of T4 immunotherapy – single dose study. SCID Beige mice were injected on day 0 with 2 million ffLuc/RFP^+ LO68 mesothelioma cells using the i.p. route. In parallel, an ErbB re-targeted ($T4^+$) or control ($P4^+$) CAR T-cell product was generated from a patient with epithelioid mesothelioma (patient 31) by retroviral transduction and expansion of the cells using IL-4. CAR T-cells (10 million) were administered by i.p. injection on day 14 (arrowed). Serial bioluminescence imaging (A) and weight measurement

(B) was performed thereafter, making comparison with PBS treated control mice (mean ± SD, n=5 mice per group). ***p =0.0003 (T4 v P4) and 0.0001 (T4 v PBS).

Fig. 7 *In vivo* anti-tumor activity of T4 immunotherapy – repeated dose study. SCID Beige mice were injected on day 0 with 2 million ffLuc/RFP$^+$ LO68 mesothelioma cells using the i.p. route. In parallel, an ErbB re-targeted (T4$^+$) or control (P4$^+$) CAR T-cell product was generated from a patient with epithelioid mesothelioma (patient 36) by retroviral transduction and expansion of the cells using IL-4. CAR T-cells or PBS were administered by i.p. injection at the arrowed time points on day 14 (10 million T-cells) and on day 21 (5 million T-cells). Serial bioluminescence imaging was performed on individual mice. Total flux (photons per second – A) and images of the animals are shown at each time point (B). Serial weight measurements were performed throughput (C). Data show mean ± SD, n=5 (T4 / PBS) or 4 (P4) mice per group. For T4 v PBS, p= 0.02 (day 21); 0.0006 (day 35) and 0.05 (day 42). For T4 v P4, p=0.06 (day 42) and 0.006 (day 49).

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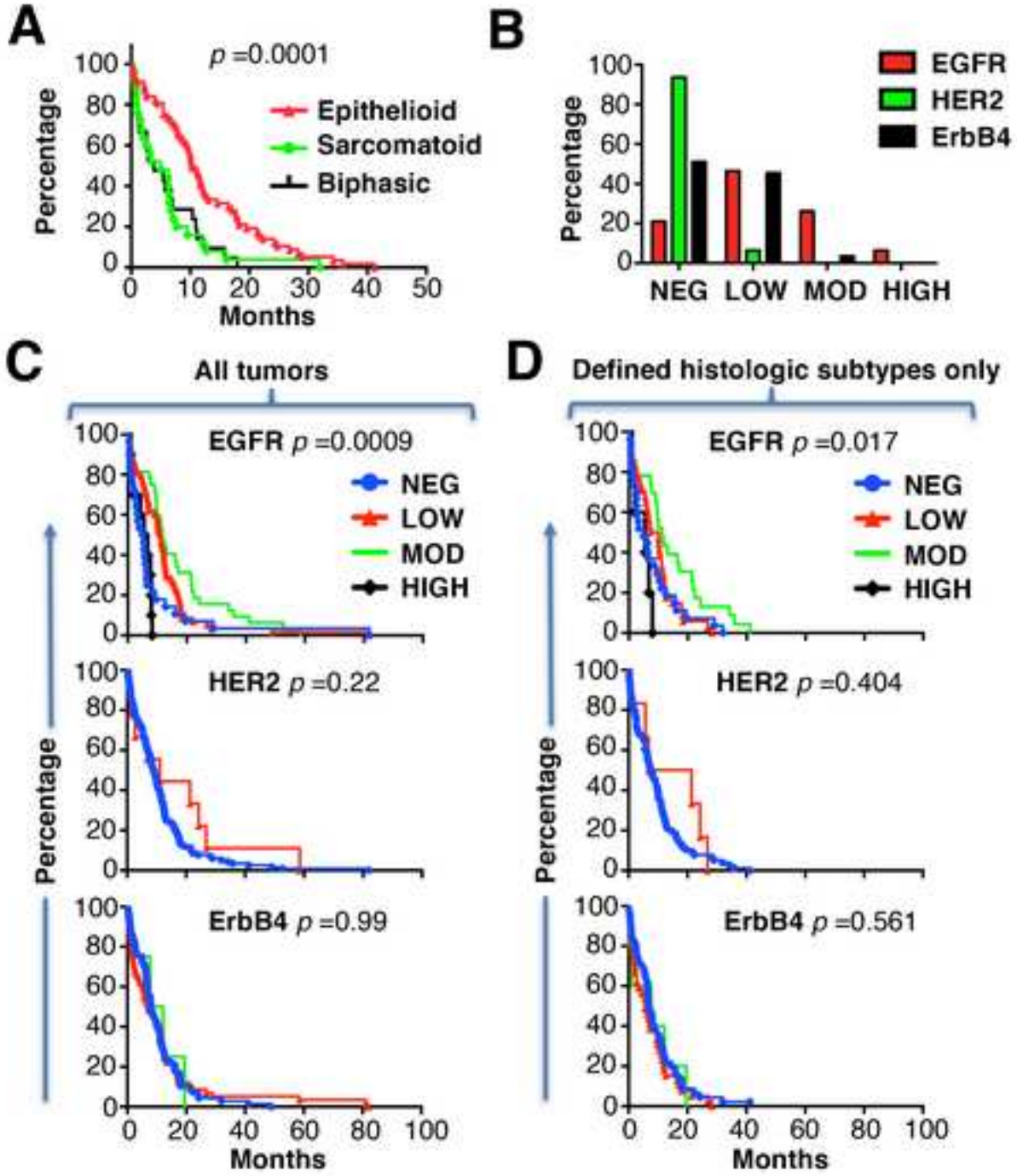

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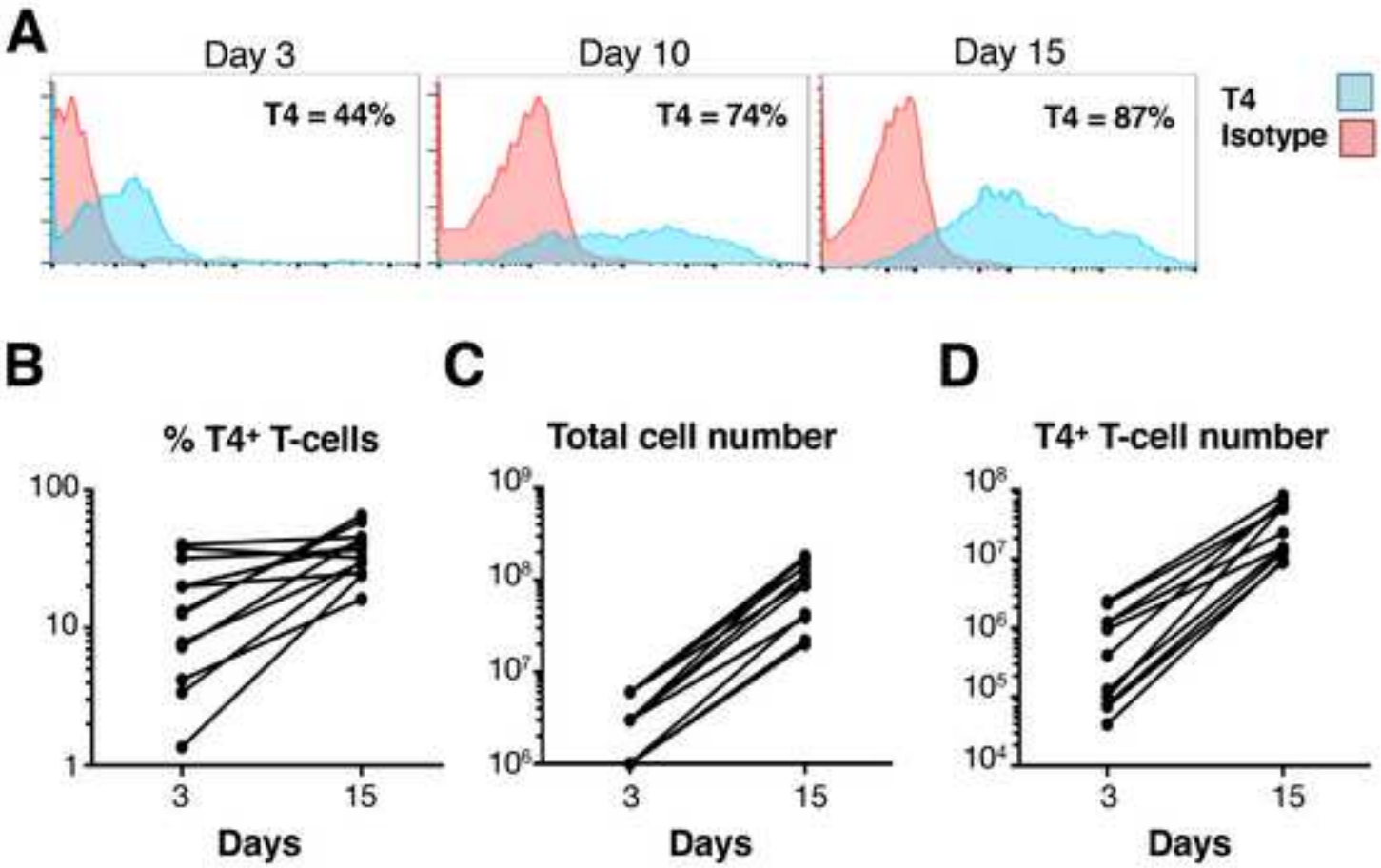

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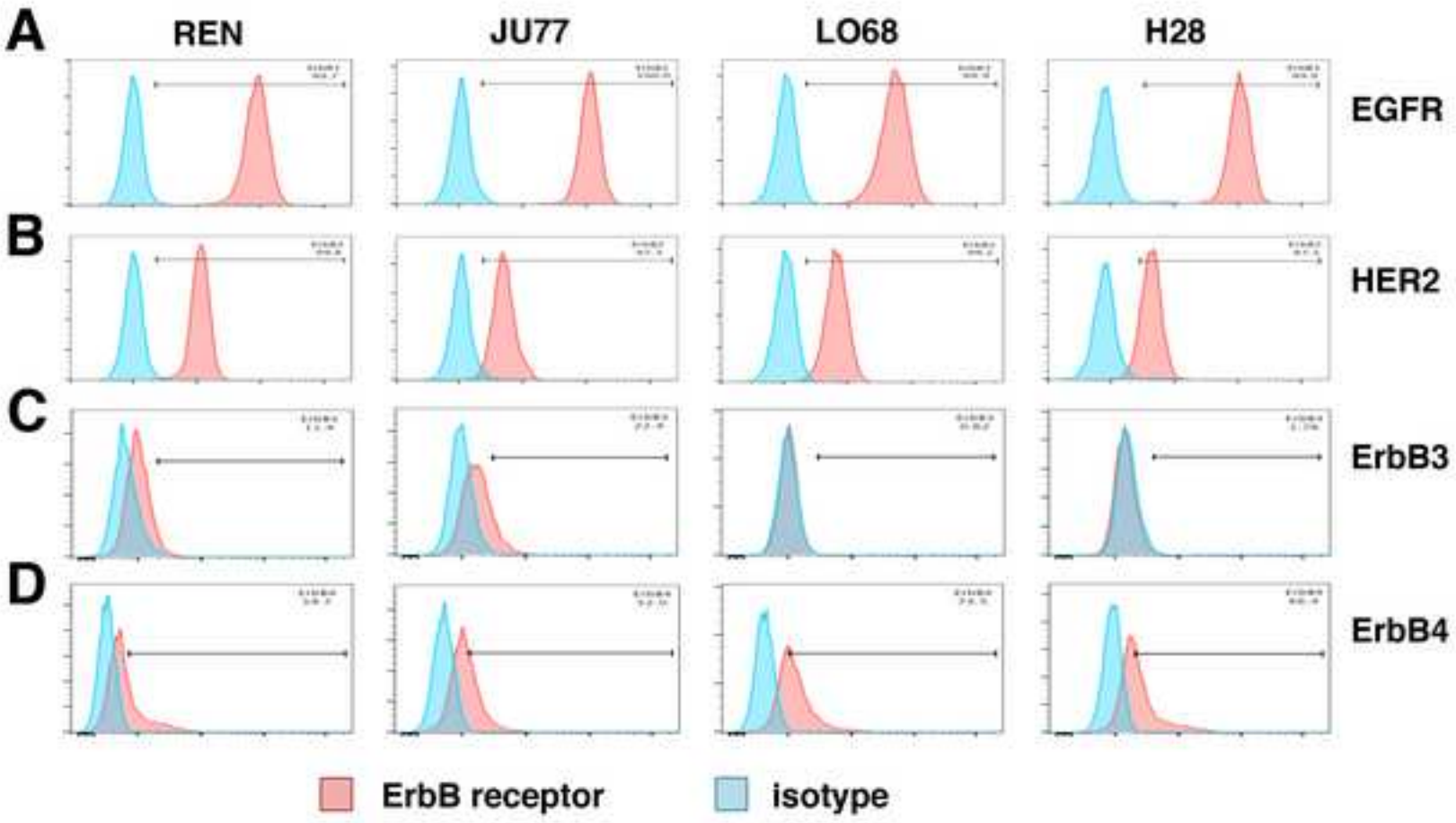

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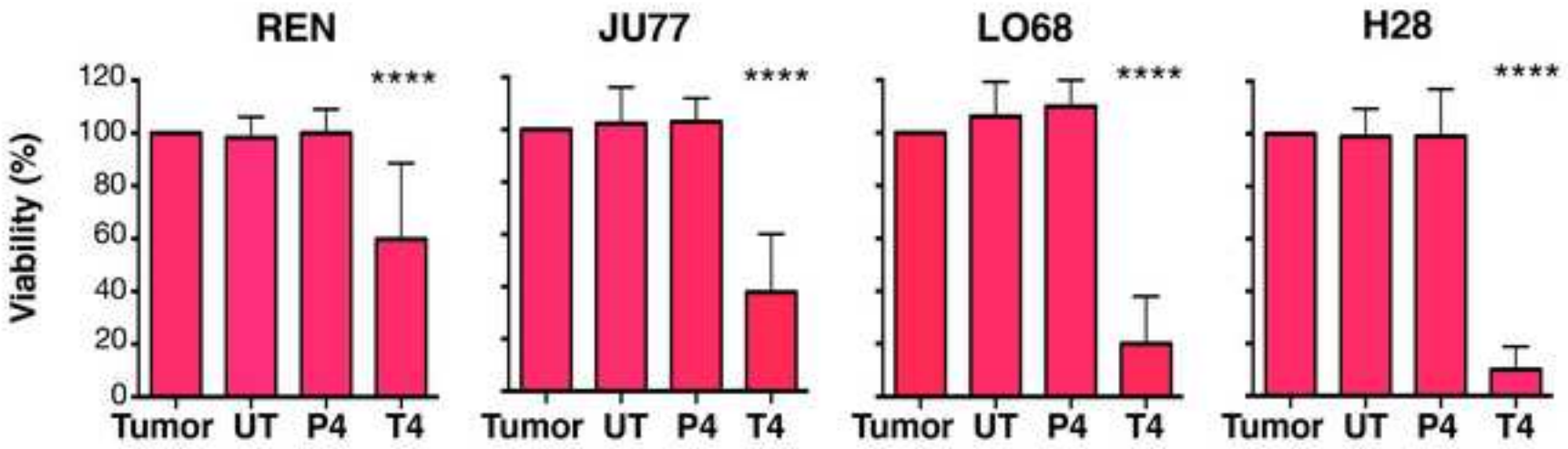

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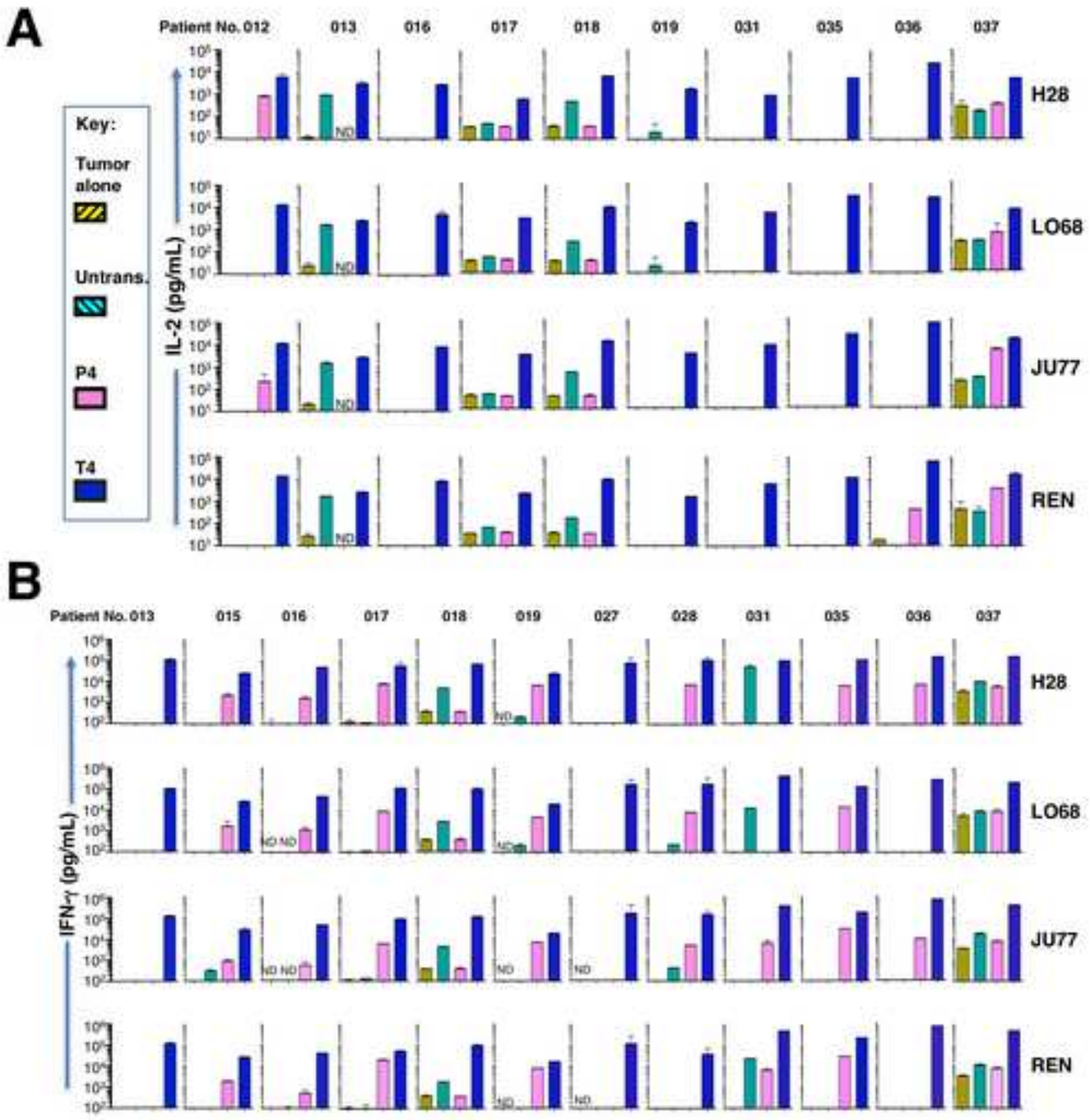

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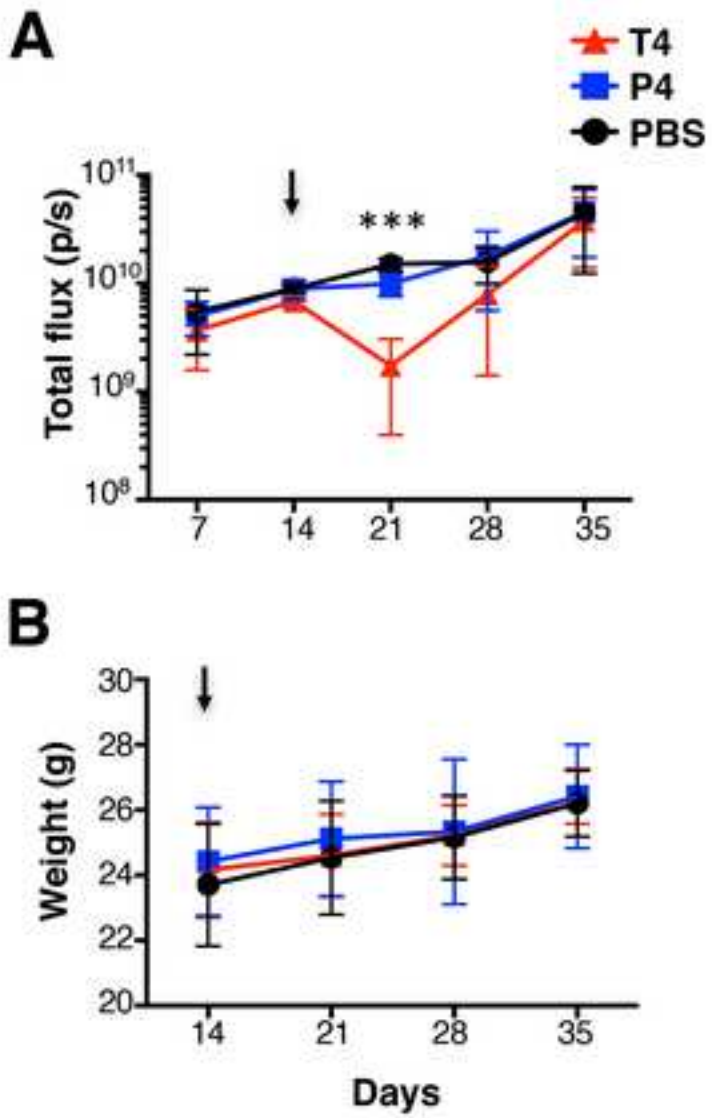

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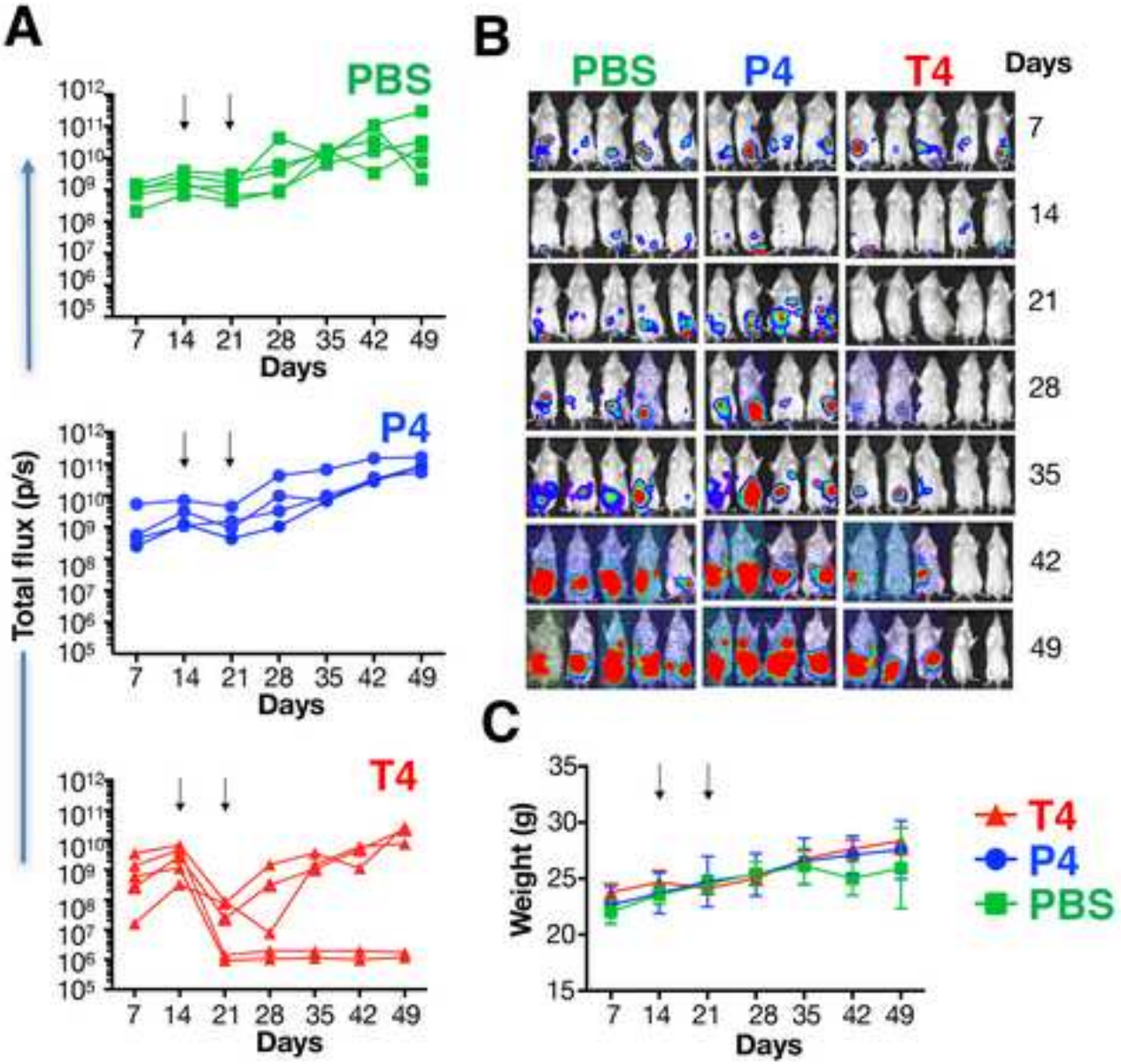

Tables

Table I ErbB receptor expression by malignant pleural mesotheliomas

	EGFr	HER2	ErbB3	ErbB4
All tumors	126 (79.2%)*	9 (6.3%)	0 (0%)	70 (49.0%)
Defined histologic subtypes				
Epithelioid	57 (89.1%)	5(8.8%)	0 (0%)	31 (62.0%)
Sarcomatoid	6 (28.6%)	0 (0%)	0 (0%)	9 (34.6%)
Biphasic	11 (61.1%)	1 (6.3%)	0 (0%)	8 (40.0%)

* number of cases (percentage shown in brackets).

Table II Median survival of patients with malignant pleural mesotheliomas

	Negative	Low	Moderate	High
All tumors				
EGFr	5.21 (28)*	11.0 (58)	11.7 (32)	6.1 (10)
HER2	9.07 (116)	11.1 (9)	NA (0)	NA (0)
ErbB4	7.98 (67)	7.17 (58)	9.96 (4)	NA (0)
Defined histologic subtypes				
EGFr	4.99 (27)	8.6 (34)	11.3 (23)	5.5 (5)
HER2	7.82 (76)	14.05 (6)	NA (0)	NA (0)
ErbB4	7.00 (44)	5.45 (39)	9.96 (4)	NA (0)
Epithelioid subtype				
EGFr	7.00 (6)	11.38 (22)	11.7 (21)	6.74 (3)

*Median survival in months (number of patients shown in brackets). NA – not applicable.

Table III Multivariate analysis of prognostic factors for patient survival

	Coefficient (b_i)*	Hazard ratio [exp(b_i)]	95% CI	*p*-value
Histology	0.712	2.037	1.402-2.96	<0.001
EGFR	0.263	1.0301	0.945-1.79	0.106
Histology	0.572	1.772	1.263-2.485	0.001
EGFR (High)	1.052	2.865	1.078-7.610	0.035

*Cox proportional hazards model

Supplementary File

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Conflicts of interests

JM is chief scientific officer of Leucid Bio, which is a spinout company focused on development of cellular therapeutic agents.