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Original Research

Pan-cancer analysis of antibody-drug conjugate targets and putative predictors of treatment response

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Abstract ***Background:*** Antibody-drug conjugates (ADCs) are a rapidly expanding class of compounds in oncology. Our goal was to assess the expression of ADC targets and potential downstream determining factors of activity across pan-cancer and normal tissues.
Materials and methods: ADCs in clinical trials (n = 121) were identified through ClinicalTrials.gov, corresponding to 54 targets. Genes potentially implicated in treatment response were identified in the literature. Gene expression from The Cancer Genome Atlas (9000+ cancers of 31 cancer types), the Genotype-Tissue Expression database (n = 19,000

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Sacituzumab govitecan;
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Agnostic drug

samples from 31 normal tissue types), and the TNMplot.com (n = 12,494 unmatched primary and metastatic samples) were used in this analysis. To compare relative expression across and within tumour types we used pooled normal tissues as reference.

Results: For most ADC targets, mRNA levels correlated with protein expression. Pan-cancer target expression distributions identified appealing cancer types for each ADC development. Co-expression of multiple targets was common and suggested opportunities for ADC combinations. Expression levels of genes potentially implicated in ADC response downstream of the target might provide additional information (e.g. *TOP1* was highly expressed in many tumour types, including breast and lung cancers). Metastatic compared to primary tissues overexpressed some ADCs targets. Single sample "*targetgram*" plots were generated to visualise the expression of potentially competing ADC targets and resistance/sensitivity markers highlighting high inter-patient heterogeneity. Off-cancer target expression only partially explains adverse events, while expression of determinants of payload activity explained more of the observed toxicities.

Conclusion: Our findings draw attention to new therapeutic opportunities for ADCs that can be tested in the clinic and our web platform (https://tnmplot.com) can assist in prioritising upcoming ADC targets for clinical development.

1. Introduction

Antibody-drug conjugates (ADCs) have emerged as a rapidly expanding new treatment modality in solid tumours. As of September 2023, six ADCs have been approved by the European Medicine Agency and/or the United States Food and Drug Administration (FDA) for solid tumours, and over 115 novel ADCs are currently being tested in clinical trials. ADCs are modern embodiments of Ehrlich's "magic bullet" applied to cancer; highly specific antibodies delivering cytotoxic cargo to cells expressing the target antigen. However, in practice, ADCs share many of the same limitations as traditional chemotherapy agents, partly due to the off-target effects of the antibody-drug conjugate (ADC) and the release of the cargo into circulation. There are very few truly cancer-type specific cell surface antigens. Solid tumour ADC targets are also expressed heterogeneously across normal tissues, although usually at a lower level than on cancer cells, and target expression is often variable within and across cancer types and tumour settings (primary versus metastasis). These target expression patterns might provide novel opportunities for broad cross-cancer therapeutic indications and to predict potential toxicities in normal tissues (on-target off-cancer). However, the number of cell surface molecules that are required for ADC-mediated cytotoxic effect (and likely toxicity) is highly variable from ADC to ADC. For example in breast cancer, ado-trastuzumab-emtansine (T-DM1) requires a high level of Human Epidermal Growth Factor Receptor 2 (HER2) protein expression for efficacy (e.g. HER2 gene (ERBB2) amplification and/or immunohistochemistry 3+ staining intensity), but fam-trastuzumab-deruxtecan-nxki (T-DXd) has efficacy also in HER2-low cancers as well (i.e. HER2/ERBB2 gene non-amplified, immunohistochemistry 1+ or 2+), and possibly even in HER2-negative (by immunohistochemistry) cancers [1–4]. This is particularly noteworthy since this huge clinical difference is essentially due to a technological ADC evolution implementing a different linker, payload and drug antibody ratio [5]. In the case of many other available ADCs, target expression is essential for clinical activity. For example, mirvetuximab soravtansine is only active in FOLR1 expressing cancers [6,7], anetumab ravtansine requires the presence of mesothelin to attain clinical responses in ovarian cancer and mesothelioma [8], and telisotuzumab vedotin is only active in MET-expressing non-small cell lung cancers [9]. In other instances, such as enfortumab vedotin (EV) in urothelial carcinoma [10] and sacituzumab govitecan (SG) in triple-negative and HER2-positive breast cancer [11,12], although clinical objective responses are more frequent in patients with higher target expression, approvals are independent of target expression because responses are also observed in patients with lower scores.

Nevertheless, a relationship between target expression level and degree of ADC activity is extremely common. Thus, high levels of target expression in a given cancer at least create the possibility of benefit from an ADC as "agnostic therapy", as supported by the DESTINY-PanTumor02 with the impressive ORR of 61.3% in patients with tumour HER2 expression of immunohistochemistry (IHC) 3+ treated with T-DXd [3].

It is also clear that there are many tumour intrinsic biological processes other than target expression levels that influence response to ADC therapy [13]. In the end, it is likely that tumour response to a particular ADC is determined by a combination of target expression level and the expression of other genes that influence internalisation, linker cleavage, and sensitivity to the cytotoxic cargo.

Our goal was to assess the target expression levels of ADCs currently in clinical development and the expression levels of molecules possibly implicated in ADC response across pan-cancer tumour types and normal human tissues. We focused our analyses on ADCs carrying cytotoxic

molecules – potential predictors of response to radionuclides and/or immune stimulators were not included. We propose a "*targetgram*" that displays the relative expression levels of the multiple ADC targets that a single cancer might express and the combined level of resistance and sensitivity genes, respectively, in the same tissue. The *targetgram* might help prioritise treatment selection for individual patients when multiple ADCs may be clinically available. Our manuscript focuses on targets against which ADCs are being tested in the clinic. However, the number of cell surface proteins on cancer cells that might serve as future ADC targets is vast, and we also developed a web tool and interface that could be used by investigators to prioritise potential targets for ADC development based on expression patterns across cancers and in normal tissues.

2. Materials and methods

2.1. Datasets

Uniformly processed RNA-Seq transcript per million (TPM) data and sample annotations for The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) were downloaded using the recount3 Bioconductor package [14]. TCGA pan-cancer reverse-phase protein array (RPPA) data were downloaded from https://gdc.cancer.gov/about-data/publications/pancanatlas (file TCGA-RPPA-pancan-clean.txt, accession date 29th March 2022). GTEx proteomic data were obtained from supplementary Table S2 of [15]. Expression data of pan-cancer metastatic and primary tumours were included in the TNMplot web tool (https://www.tnmplot.com/) [16].

Gene array datasets from TNMplot.com – a web-interactive interface that incorporates gene arrays from the Gene Expression Omnibus of the National Center for Biotechnology Information (NCBI-GEO), RNA-seq data from TCGA, Therapeutically Applicable Research to Generate Effective Treatments (TARGET), and GTEx [16] – were used for analyses of metastases. A sample size of each dataset is detailed in Supplementary Tables 1, 2, and 3.

2.2. Bioinformatics pipeline

TPM values of TCGA and GTEx RNA-Seq data were $\log_2$-transformed after adding a constant value of 1. Principal component analysis (PCA) was performed for TCGA cancer types with at least 30 tumour-matched normal tissues and 30 normal samples of the corresponding organ in GTEx. For downstream analyses, only TCGA samples annotated as "Primary tumour" were selected. In GTEx, samples with missing tissue annotation were discarded.

Gene array samples have been normalised using the MAS5 method by the Affy Bioconductor R package, followed by a second scaling normalisation, in which all the mean expressions for each set were set to 1000.

When comparing tumours and normal samples, the level of over-expression of a gene was defined as the percentage of samples in each cancer type with an expression above the 80th percentile of the gene expression distribution across all normal samples. The same approach was applied for the identification of over-expressors in each normal tissue compared to the overall expression in normal tissues. To evaluate over-expression in each tumour type compared to the overall pan-cancer expression, the 80th percentile of the gene distribution was calculated across all TCGA tumour types. Differential expression analysis and $\log_2$ fold changes were assessed by limma 16. P-values were corrected for multiple testing according to the Benjamini-Hochberg false discovery rate. Correlation between continuous variables was assessed by Spearman's correlation coefficients. Hierarchical clustering was performed using Euclidean distance and Ward linkage.

To generate TCGA single-sample-level *targetgrams* we converted the expression value of each target in its corresponding quantile using the pan-cancer gene distribution as the reference. The summary of resistance and sensitivity markers expression was evaluated as the median quantile of the genes in the two categories.

All analyses were performed in R version 4.2.1 with Bioconductor version 3.15.

2.3. Identification of ADCs in clinical testing and literature search for molecular predictors of ADC response

Between 7th March 2022 and 26th September 2023, clinicaltrials.gov was accessed multiple times with different terms, including, for example, "antibody-drug conjugate", "trastuzumab deruxtecan", "trastuzumab emtansine", "sacituzumab govitecan", "enfortumab vedotin", "mirvetuximab soravtansine", "tisotumab vedotin" to identify clinical trials testing ADCs. Trials testing ADCs in non-solid tumours were excluded from analyses. The full search history is reported in the Supplementary Table 4. We included actively recruiting, completed, as well as discontinued and terminated trials. The retrieved trials were annotated with NCT number, tumour site, disease setting (e.g. advanced/metastatic), study phase, ADC name, ADC target gene, ADC type (monospecific or bispecific), second ADC target gene in case of bispecific ADC, and payload class. Phase 1|2 trials were annotated as phase 2 trials, phase 2|3 trials were annotated as phase 3 trials.

The selection of predictors of ADC activity was manually curated. Google Scholar served as research engine and was searched for terms "antibody-drug conjugate", "mechanism of resistance", "resistance", "sensitivity", "toxicity", "predicts", "screen", "*in vitro*", "trastuzumab emtansine", "T-DM1″, "trastuzumab

deruxtecan", "trastuzumab", "toxicity", "clathrin-mediated endocytosis", "caveolae", "cathepsins", "bystander", "topoisomerase I", "camptothecin", "vedotin" in various combinations. Review articles served for cross-referencing. Selection criteria for inclusion of potential predictors were i) demonstrated mechanistic impact in pre-clinical models, including cell lines and/or mouse models, ii) being part of a pathway that has a demonstrated role in mediating ADC activity, e.g. clathrin-mediated endocytosis, iii) being a determinant of response to naked antibodies, e.g. trastuzumab, if the considered gene has an impact on intracellular trafficking or cell surface localisation of target antigens.

2.4. Web-tool

The web interactive interface has been constructed as a spin-off of TNMplot.com (https://tnmplot.com) [16]. Gene arrays of the NCBI-GEO, RNA-seq from TCGA, TARGET, and GTEx repositories constitute the datasets available for analyses. By accessing the Gene expression comparison tab, and selecting the multi-gene analysis option, the user can plot gene expression profiles of input genes in normal tissues, primary solid tumours, and metastases. The input consists of one or more gene symbols and one tissue of interest (e.g. breast, cervix, endometrium, etc.), the output can be a density or a box plot, at the user's preference. In addition, the user can plot a dot plot showing the differential expression of the primary tumour and normal tissues which is represented by the colour range of each dot, p-values are inversely proportional to the sizes of the points. Users can access this feature by navigating to the pan-cancer tab and selecting the pan-cancer heatmap option. Also, the user can further investigate the expression profile of their genes of interest by using the "targetgram" option (found under the Gene expression comparison tab), which represents the mean expression values of the target genes in the subselected tissue type.

3. Results

3.1. ADCs under clinical development in solid tumours

We identified 121 ADCs, targeting 54 distinct cell surface molecules, which are tested in 545 clinical trials in solid tumours. The most frequent targets of ADCs in clinical development were HER2/*ERBB2* (224 trials), Trop2/ *TACSTD2* (69 trials), Folate Receptor-α (FOLR1) (32 trials), EGFR (26 trials), and Nectin-4 (25 trials). The most studied ADCs were T-DM1 (99 trials), T-DXd (50 trials), SG (46 trials), EV (23 trials), patritumab deruxtecan (12 trials), MRG002 (12 trials), and rovalpituzumab tesirine (12 trials). (Supplementary Fig. 1, Supplementary Table 5). The most common ADC payloads were auristatin derivatives (185 trials), maytansinoids (154 trials), and camptothecin analogues (136 trials).

3.2. ADC target expression is heterogeneous within and across cancer types

Since TCGA and GTEx RNA-Seq data derive from two different studies we first assessed whether strong batch effects could affect data analysis. PCA showed that TCGA tumours and GTEx normal samples cluster separately (Supplementary Fig. 2).

However, normal samples derived from TCGA patients clustered with GTEx samples or formed an intermediate cluster between TCGA tumours and GTEx normal. In case of strong batch effects, one would expect a complete separation of TCGA and GTEx independently from the sample type. These results indicate that the overall transcriptome profiles are dominated by differences between normal and tumour samples instead of batch effect, despite we cannot exclude that the separation of TCGA tumours and GTEx normals may be amplified by technical reasons.

First, we assessed the correlation between protein and mRNA levels for the 54 ADC targets in the GTEx and TCGA datasets. For most targets, the protein and mRNA expression levels were positively correlated except for ENPP3 and LIV-1/*SLC39A6*, which showed negative correlation between protein and mRNA levels (Supplementary Fig. 3).

To illustrate relative expression differences in ADC target expression across cancer types and simultaneously capture the cancer specificity of the target, we plotted target expression as a fold change of mean mRNA expression in a given cancer type compared to all normal tissues in the GTEx. We observed widespread heterogeneity in target expression across and within cancers; furthermore, most cancers expressed multiple targets at different levels (Fig. 1A).

The most broadly overexpressed ADC targets across cancer types were *EFNA4*, *MET*, B7-H3/*CD276*, *NECTIN4*, and *PTK7*. Pancreatic, lung, breast, oesophageal, and head and neck cancers were the richest ADC targets. Uveal melanoma, adrenocortical carcinomas, and pheochromocytoma had the fewest overexpressed targets. When target expression in each cancer type was compared to all other cancers (rather than relative to normal tissues), breast, lung, pancreatic, and oesophageal tumours had the highest expression levels of several ADC targets (Supplementary Fig. 4).

Within each cancer type the percentage of samples with gene over-expression was highly variable, depending on the ADC target. Certain ADC targets were over-expressed in most samples of individual cancer types. For instance, 100% of ovarian tumours (OV) had *FOLR1* expression above the 80th percentile of its expression in normal tissues. Target expression distributions showed different patterns in normal tissues and in

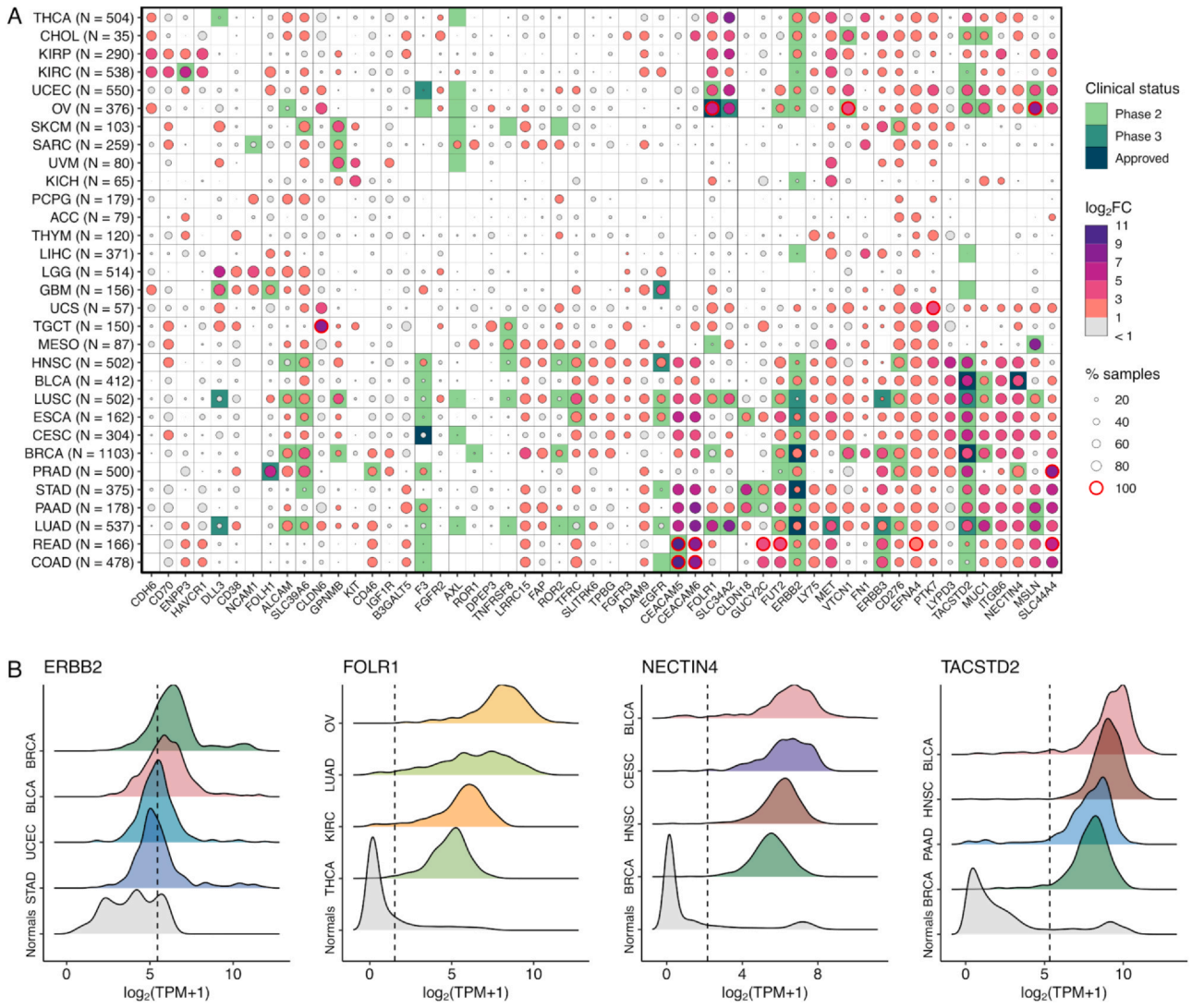

Fig. 1. Antibody-drug conjugate (ADC) targets are shared across solid tumours and heterogeneously expressed within cancer types. A) ADC target expression in primary tumours from The Cancer Genome Atlas (TCGA) and normal tissues from the Genotype-Tissue Expression (GTEx) repository. ADC targets are shown on the x-axis and cancer types on the y-axis. Fold-change (FC) of mean mRNA expression values (Log_2[TPM+1] mRNA counts) of ADC targets in a given cancer type relative to all normal tissues in the GTEx. Darker dot colours indicate higher expression in cancer. Dot size is proportional to the percentage of tumour samples exceeding an expression threshold defined as the 80th centile of expression seen in pooled normal tissues in the GTEx. The background colour of the squares indicates the clinical development stage in the given cancer type (white indicates phase 1 trials, light green phase 2 trials, darker shades of green phase 3 trials and approved indications, respectively). Annotations were made according to disease-specific information from the participation criteria section of each trial: generic terms as "solid tumour", or "other solid tumour" did not qualify for annotation of all TCGA types. B) mRNA expression distribution of 4 representative ADC targets in exemplary cancer types and in GTEx normal tissues. Vertical dashed lines indicate the 80th centile in normal pooled tissues for each target. ACC, adrenocortical carcinoma, BLCA Bladder Urothelial Carcinoma, LGG Brain Lower-Grade Glioma, BRCA Breast invasive carcinoma, CESC Cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL Cholangiocarcinoma, COAD Colon adenocarcinoma, ESCA Oesophageal carcinoma, GBM Glioblastoma multiforme, HNSC Head and Neck squamous cell carcinoma, KICH Kidney Chromophobe, KIRC Kidney renal clear cell carcinoma, KIRP Kidney renal papillary cell carcinoma, LIHC Liver hepatocellular carcinoma, LUAD Lung adenocarcinoma, LUSC Lung squamous cell carcinoma, MESO Mesothelioma, OV Ovarian serous cystadenocarcinoma, PAAD Pancreatic adenocarcinoma, PCPG Pheochromocytoma and Paraganglioma, PRAD Prostate adenocarcinoma, READ Rectum adenocarcinoma, SARC Sarcoma, SKCM Skin Cutaneous Melanoma, STAD Stomach adenocarcinoma, TGCT Testicular Germ Cell Tumours, THYM Thymoma, THCA Thyroid carcinoma, UCS Uterine Carcinosarcoma, UCEC Uterine Corpus Endometrial Carcinoma, UVM Uveal Melanoma.

different cancer types (Fig. 1B). For illustrative purposes, expression profiles of targets of approved ADCs are shown in Fig. 1B and Supplementary Fig. 5.

The expression of multiple ADC targets on same cancer raises the possibility of improving therapeutic effects by developing bispecific ADCs or combining

ADC therapies. We examined pair-wise co-expression correlations and identified several potentially clinically actionable associations (Supplementary Fig. 6). For example, *NECTIN4* is highly correlated with Trop2/*TACSTD2*; HER2/*ERBB2* is correlated with *ERBB3*, Trop2/*TACSTD2*, and *NECTIN4*.

At the target level, the largest differential expression between cancer and healthy tissues was seen for *CEACAM5* in colorectal cancers, *CEACAM6* in lung, colorectal, and pancreatic adenocarcinomas, mesothelin/*MSLN* in ovarian carcinoma and mesothelioma, and *SLC34A2* in lung adenocarcinoma and thyroid carcinoma.

3.3. The ADC target landscape in metastases is different from primary tumours

During tumour progression the transcriptomic programme can change, leading to the possible loss of expression of certain ADC targets or to the up-regulation of new ones. Therefore, we compared ADC mRNA expression in multiple cohorts of primary tumours and metastatic lesions collected in TNMplot [16] (Fig. 2).

We found that 100% (n = 49, 5 genes being excluded in the quality-control processing of data) of ADC targets were differential expressed between metastases and primary tumours in at least one cancer type. Across the assessed cancer types, the percentage of ADC targets differentially expressed ranged from 45% (n = 22) in the liver to 92% (n = 45) in the oesophagus. Among the targets of currently approved ADCs, Trop2/*TACSTD2* displayed higher expression in metastases compared to primary tumours in pancreatic, skin, and oesophageal cancers, and *FOLR1* in ovarian and breast cancers. *MSLN* expression was also higher in metastatic ovarian cancer, B7-H4/*VTCN1* in metastatic breast cancer, and *MUC1* in metastatic pancreatic and breast cancers.

3.4. Treatment-associated adverse events are partly explained by target expression in normal tissues

Since none of the current ADC targets are truly cancer-specific, we examined if on-target off-cancer effects of ADCs could explain treatment-emergent adverse events of the 6 ADCs that are currently approved for the treatment of solid tumours. Target expressions in normal tissues were obtained from the GTEx database and relative expression levels by organ sites are shown in (Supplementary Fig. 7).

The results suggest that there is only a moderate association between on-target off-cancer effects of ADCs and treatment-related adverse events (safety profiles were taken from FDA package inserts, details in Supplementary Tables 6 and 7). For example, HER2/*ERBB2* is most expressed in the prostate, nerve and thyroid relative to all normal adult tissues, yet these tissues are not affected by treatment-emergent adverse events of anti-ERBB2/HER2 ADCs. HER2/*ERBB2* is also expressed in the skin and salivary gland, which may explain why rash and stomatitis are associated with both T-DM1 and T-DXd. On the other hand, Trop2/*TACSTD2* is highly expressed in the salivary gland, lung, prostate, and skin, yet only oral mucosa, lung and skin-related toxicities have been reported. As a last example, *NECTIN4* levels are high in the skin, which may explain the frequent cutaneous adverse events (54%) seen with EV.

3.5. Expression of genes associated with sensitivity or resistance to ADCs

We identified 59 genes as potentially implicated in ADC response through a literature search, 14 of these were associated with resistance and 45 with sensitivity (Supplementary Table 8).

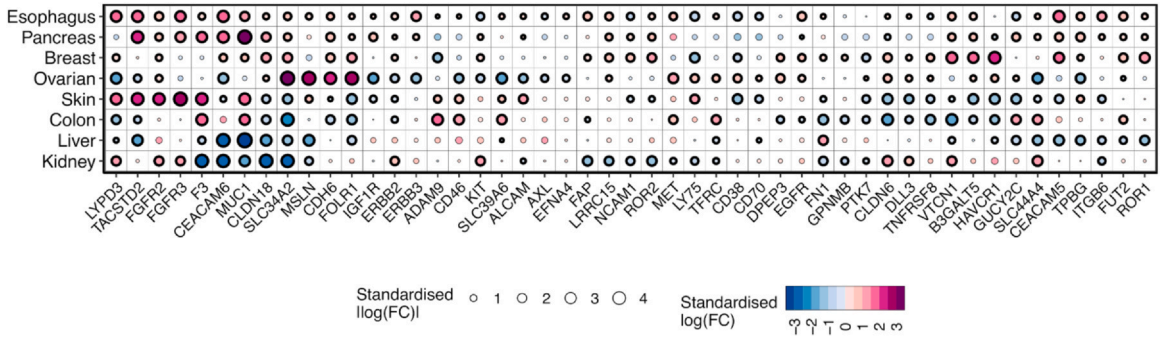

Fig. 2. ADC targets are differently expressed in metastases and primary tumours. Expression of target genes was compared between metastases and primary tumours using data from TNMplot.com. Primary and metastatic samples are not patient-matched. Histologic subtypes were aggregated by anatomical site of origin to reach an adequate sample size (e.g. 'skin' includes primary and metastatic melanoma, skin squamous cell carcinoma and other rare skin cancer histologies). Low-expressed genes were filtered. ADC target genes are shown on the x-axis, and anatomical sites of origin are shown on the y-axis. Dot size indicates the absolute value of fold-change (FC) differences in mean Log_2(TPM+1) mRNA counts between metastases and primary tumours. Directionality is given by dot colour. Darker blue dot colour indicates lower expression in metastases, darker red dot colour indicates higher expression in metastases.

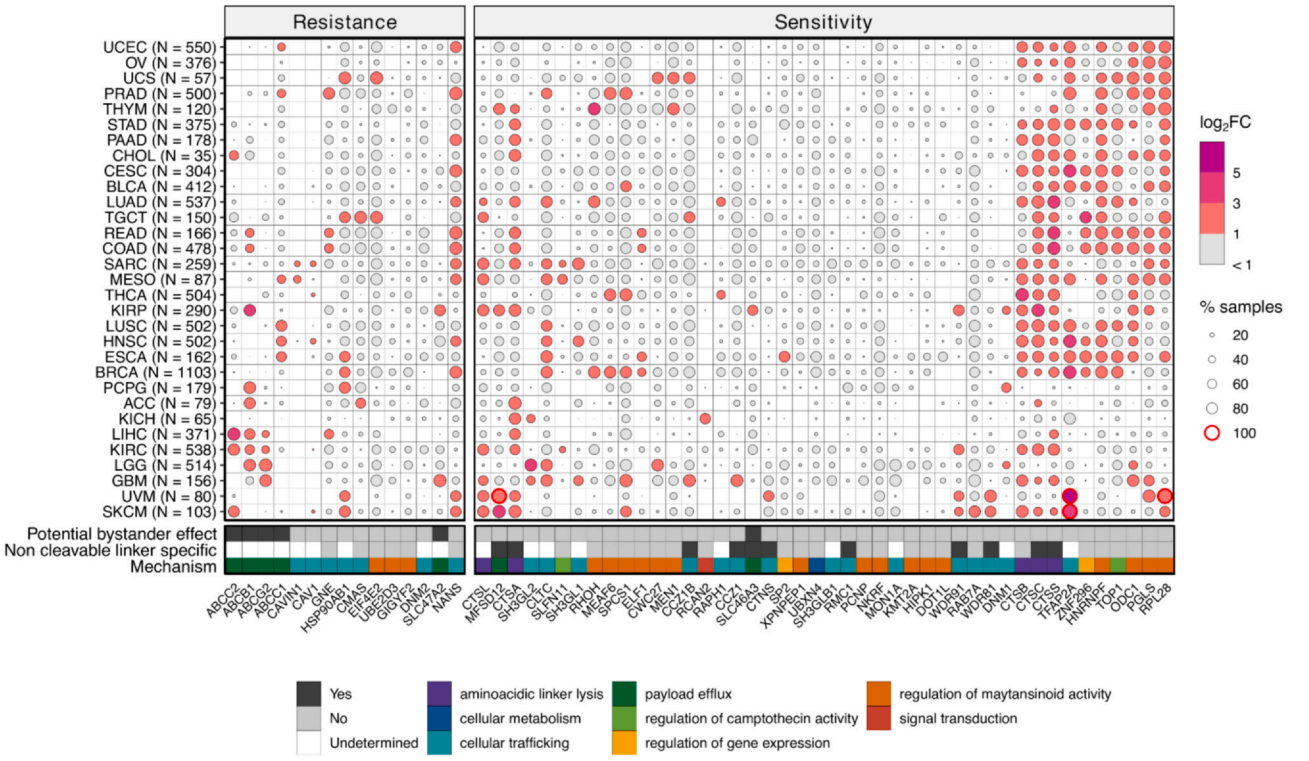

Fig. 3. Genes implicated in ADC response or resistance are heterogeneously expressed across and within cancer types. Relative expression levels of 59 genes implicated in ADC activity (15 associated with resistance and 44 with sensitivity) are shown. Genes are listed on the x-axis and annotated with broad mechanism of action, cleavable-linker specific activity, and potential influence on bystander cytotoxic effect of the cargo. Dots indicate the fold-change (FC) differences in mean Log_2 (TPM+1) mRNA counts between a given TCGA primary cancer type and all normal tissues in the GTEx. Darker dot colours indicate higher expression in the given cancer type. Dot size is proportional to the percentage of samples exceeding an arbitrary threshold value defined at the 80th centile of expression in the pooled GTEx dataset. ACC, Adrenocortical carcinoma, BLCA Bladder Urothelial Carcinoma, LGG Brain Lower-Grade Glioma, BRCA Breast invasive carcinoma, CESC Cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL Cholangiocarcinoma, COAD Colon adenocarcinoma, ESCA Oesophageal carcinoma, GBM Glioblastoma multiforme, HNSC Head and Neck squamous cell carcinoma, KICH Kidney Chromophobe, KIRC Kidney renal clear cell carcinoma, KIRP Kidney renal papillary cell carcinoma, LIHC Liver hepatocellular carcinoma, LUAD Lung adenocarcinoma, LUSC Lung squamous cell carcinoma, MESO Mesothelioma, OV Ovarian serous cystadenocarcinoma, PAAD Pancreatic adenocarcinoma, PCPG Pheochromocytoma and Paraganglioma, PRAD Prostate adenocarcinoma, READ Rectum adenocarcinoma, SARC Sarcoma, SKCM Skin Cutaneous Melanoma, STAD Stomach adenocarcinoma, TGCT Testicular Germ Cell Tumours, THYM Thymoma, THCA Thyroid carcinoma, UCS Uterine Carcinosarcoma, UCEC Uterine Corpus Endometrial Carcinoma, UVM Uveal Melanoma.

Genes implicated in ADC internalisation, linker lysis, and endosomal trafficking are numerous, and there is a lack of evidence on the clinical predictive value of their mRNA levels. Despite this lack of evidence, we conducted an exploratory analysis of their mRNA profiles in solid tumours. Relative expression levels of these genes are shown for each primary cancer type in Fig. 3.

Similar to the ADC targets, potential predictors of response also have highly variable expression levels across and within cancer types.

Cathepsins are peptidases involved in the lysis of aminoacidic linkers (e.g. valine-citrulline). Cathepsins B and L1 (*CTSB* and *CTSL*) are highly expressed in many cancer types (e.g. mesothelioma, lung adenocarcinoma etc.). Interestingly, bladder cancer that benefits from T-DXd [3] and EV [10] was not among the cancer types that showed high levels of cathepsin B and L expression (Fig. 3), indicating that relatively low levels of cathepsins may be sufficient for ADC activity, or other proteolytic enzymes mediate cleavage[17].

4 out of 6 approved ADCs in solid tumours leverage camptothecin/irinotecan derivatives (e.g. govitecan, deruxtecan), that are topoisomerase 1 (*TOP1*) inhibitors, and maytansinoids (e.g. mertansine/DM1) that inhibit microtubule polymerisation. High *TOP1* expression has been proposed as a marker of sensitivity to camptothecin analogues [18]. Gastrointestinal tract, breast, and lung adenocarcinomas are among the cancers with the highest expression of *TOP1* making these cancers attractive targets for TOP1-inhibitor-carrying ADCs. Some predictors of maytansinoid activity (*RPL28, PGLS, ODC1, HNRNPF*) were highest in cancers of the prostate, endometrium, biliary tract, colon/rectum, and mesothelioma, other predictors of

maytansinoid activity (*DOT1L, HIPK1, KMT2A, NKRF, and PCNP*) showed no overexpression in all cancer types, while others (*MEN1, CWC27, ELF1, SPCS1, MEAF6, RHOH*) had a generally low expression among cancer types with isolate outliers, e.g. breast cancer, which showed overexpression of the latter four genes. None of the genes associated with resistance to maytansinoids (*GIGY2F, UBE2D3, EIF4E2*) was overexpressed in all cancer types, with the only exception of *EIF4E2,* which was high in testicular cancer and uterine carcinosarcoma.

We also examined the expression of genes associated with sensitivity or resistance to ADCs in metastases relative to primary tumours (Fig. 4).

CTSB, which hydrolyses peptide linkers, as well as cathepsins S and A (*CTSS* and *CTSA*), that hydrolyse non-cleavable linkers (as in T-DM1), showed decreased expression in metastases compared to primary breast cancers, while *CTSL,* which hydrolyses the peptide linker of T-DXd, showed increased expression in this tumour type. As of putative predictors of sensitivity to payloads, expression of *TOP1* was modestly increased in metastatic ovarian cancers. Expression of genes influencing the activity of maytansinoids was highest in kidney metastatic cancers. Transmembrane transporters MDR1/*ABCB1*, which mediates resistance to emtansine and vedotin [19,20], and *ABCC2*, which mediates resistance to trastuzumab emtansine *in vitro* [19], were upregulated in breast cancer metastases, while BCRP/*ABCG2*, which mediates camptothecin efflux and contributes to the bystander effect seen with T-DXd is upregulated in metastases relative to primary breast cancer.

3.6. ADC toxicities are in part recapitulated by determinants of payload activity in healthy tissues

Free cytotoxic cargo is detectable in the circulation after ADC therapy due to less than perfect linker stability, the release of the drug from dying tumour cells, and active membrane transport (i.e. bystander effect). This contributes more to the broader adverse event spectrum of ADCs than what target expression on normal tissues alone would predict. We speculated that expression levels of genes associated with sensitivity or resistance to ADCs in normal tissues could also predict adverse events of ADCs. High *CTSL* and *TOP1* were found in the bone marrow and lung, which may explain the frequent haematological toxicities and occasional pneumonitis seen with trastuzumab deruxtecan therapy. Predictors of sensitivity to maytansinoids were most overexpressed in the bone marrow, which could contribute to the frequent haematological toxicities of T-DM1 (Supplementary Fig. 8) [21].

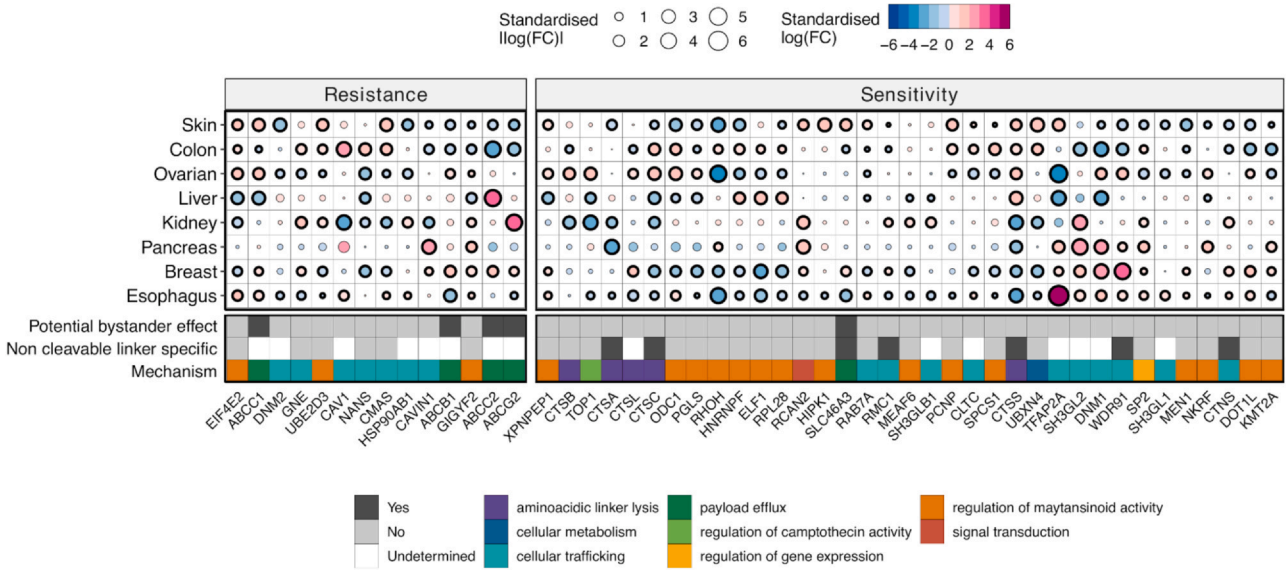

Fig. 4. Genes associated with ADC response are differently expressed in metastases and primary tumours. Expression levels of genes implicated in ADC activity were compared in metastases and primary tumours using data from TNMplot.com as described in Fig. 2. Histotypes were aggregated according to the anatomical site of origin of tumours to reach adequate sample size. Log_2(TPM+1) mRNA counts were used for analysis. Low-expressed genes were filtered and excluded from analyses. Determinants of ADC activity are plotted on the x-axis and classified according to the mechanism, cleavable-linker specificity, and potential implication into the bystander effect. Genes putatively associated with sensitivity and resistance are clustered separately. Anatomical sites of origin of tumours are plotted on the y-axis. Differential gene expression (DGE) was defined by fold-change (FC) of mean values of genes in metastases versus the mean value of primary tumours of the same anatomical site. Dot size indicates the absolute value of fold-change (FC) differences in mean Log_2 (TPM+1) mRNA counts between metastases and primary tumours. Directionality is given by dot colour. Darker blue dot colour indicates lower expression in metastases, darker red dot colour indicates higher expression in metastases.

3.7. ADC targets and expression of sensitivity and resistance markers in breast cancer subtypes

To further assess tumour heterogeneity within tumour types, we assessed the expression of ADC targets and potential biomarkers of response across breast cancer clinicopathological subtypes.

Most ADC targets showed similar expression profiles in the 4 distinct breast cancer subtypes (HER2+/ER+, HER2+/ER-, HER2-/ER+, and HER2-/ER- [a.k.a. TNBC], criteria for definition are specified in Supplementary Table 9) (Fig. 5).

However, HER2+ breast cancers showed homogenously higher relative expression of *ITGB6* and *LRRC15*, making these cancers potential targets for SGN-B6A and ABBV85/samrotamab vedotin therapies, respectively. ER-negative cancers had an additional set of highly expressed targets that were not shared with ER+ cancers including *SLC34A2*, *FOLR1*, *MSLN*, *FOLH1*, *MET*, *LY75*, *KIT*, and *CLDN6*. ER+HER2- tumours that are the least

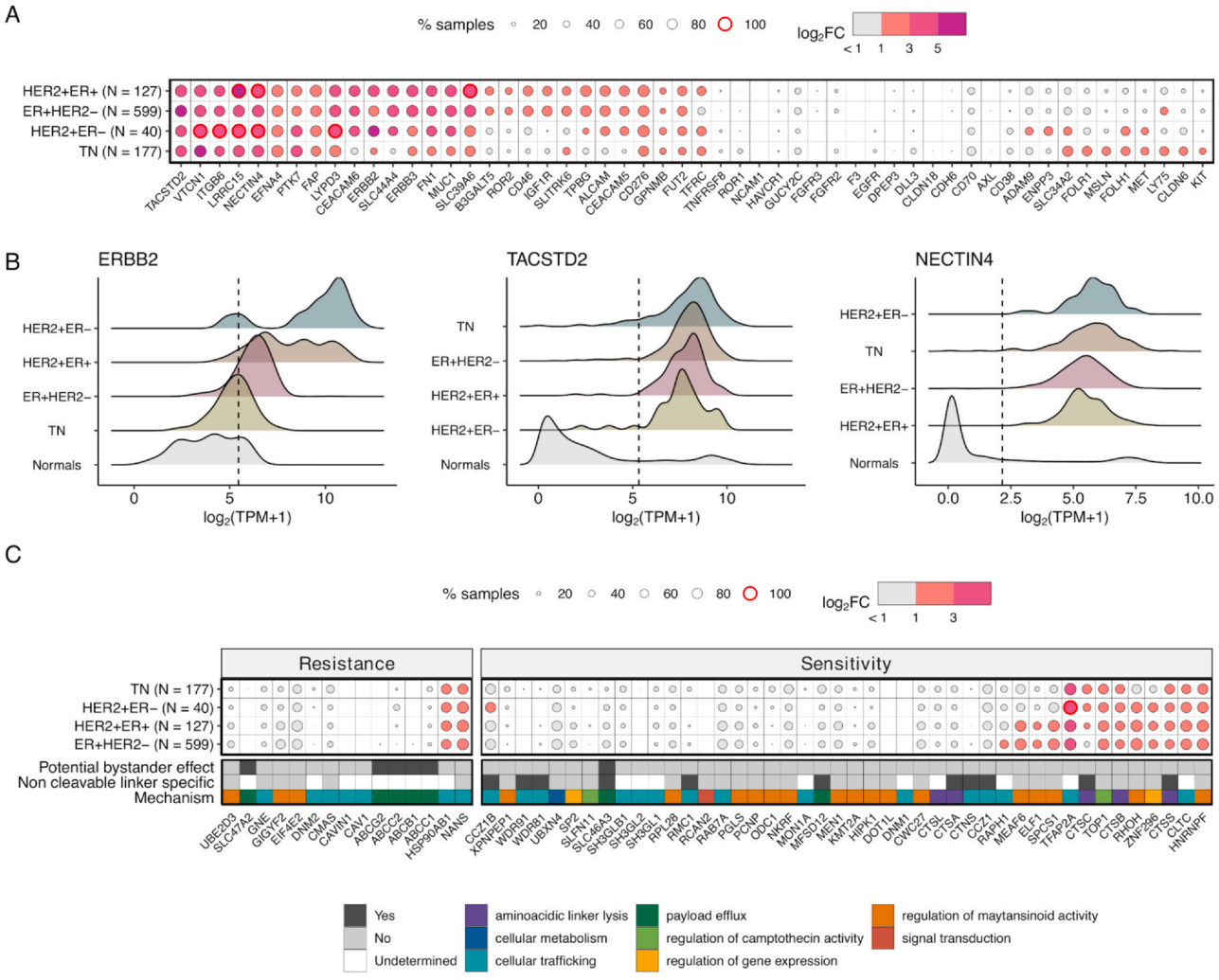

Fig. 5. Breast cancer clinicopathological subtypes have similar sensitivity profiles to most ADCs. A) Expression of ADC target genes in subtypes of breast cancers. Log_2(TPM+1) mRNA counts were analysed. ADC target genes are shown on the x-axis, and subtypes are shown on the y-axis. Differential gene expression (DGE) was defined by fold-change (FC) of mean values of ADC targets in each subtype versus the mean value of the whole GTEx. Darker dot colours indicate higher differential expression. Dot size is proportional to the percentage of samples exceeding an arbitrary threshold value defined as the 80th centile of the whole GTEx dataset. B) Expression distribution of 3 ADC targets in GTEx normal tissues and breast cancer subtypes. Vertical dashed lines indicate thresholds defined as the 80th centile of target expression in the pooled normal tissues. C) expression levels of genes associated with ADC response in breast cancers and normal tissues. Log_2(TPM+1) mRNA counts were used for analysis. Low-expressed genes were filtered and excluded from analyses. Genes involved in ADC activity are listed on the x-axis and classified according to the mechanism, cleavable-linker specific activity, and potential implication into the bystander effect. Results are shown separately for genes associated with sensitivity and resistance, respectively. Differential gene expression (DGE) was defined by fold-change (FC) of mean values of genes in each subtype from the TCGA versus the mean value of the whole GTEx. Darker dot colours indicate higher differential expression. Dot size is proportional to the percentage of TCGA tumour samples exceeding an arbitrary threshold value defined at the 80th centile of the whole GTEx dataset.

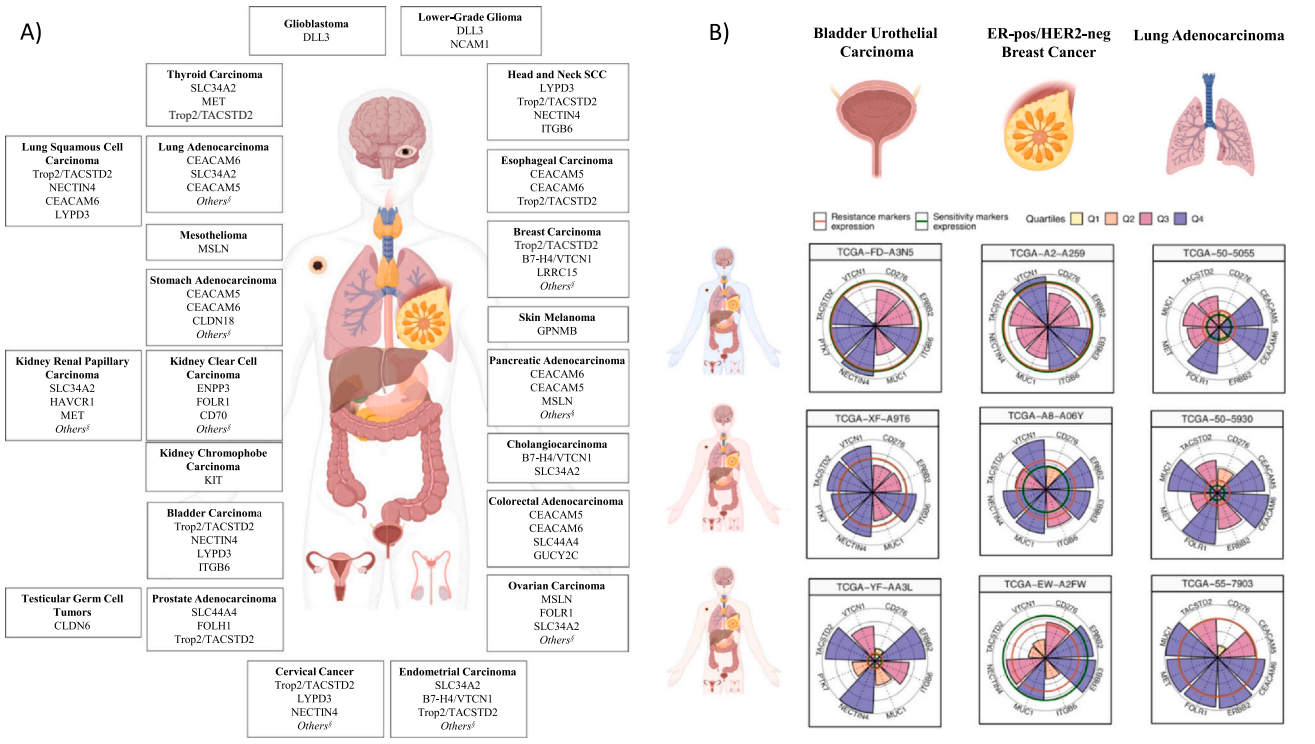

Fig. 6. Pan-cancer and patient-level heterogeneity in ADC targets. A) Expression profiles of ADC targets of primary tumours from TCGA were compared with healthy tissues from the GTEx repository, as described in Fig. 1. A fold-change (FC) of mean mRNA expression values (Log_2 [TPM+1] mRNA counts) greater than 4 was established as a threshold to sub-select most-relevant ADC targets per each tumour type. Targets with expression values greater than the threshold are listed inside the boxes and ranked in decreasing order of differential expression. (§) Others means that more than 4 targets had a FC greater than 4. B) To provide an exemplification of the inter-patient heterogeneity within histology, the "*targetgrams*" of 9 individual cancers representing 3 different cancer types are presented depicting the relative expression levels of eight ADC targets. Radial length is proportional to the expression value of each gene in each sample relative to all the other samples of TCGA. Expression values are represented as quartiles indicated by concentric circles. The red circle indicates the median expression of all resistance genes taken together; the green circle indicates the median of all sensitivity genes taken together. Abbreviations: SCC squamous cell carcinoma.

sensitive to traditional chemotherapies had high expression of many ADC targets, including, for example, B7-H3/*CD276*, B7-H4/*VTCN1*, *ERBB3*, and *LRRC15*. Among the genes implicated in ADC response, many were highly expressed (e.g. *TOP1*, *CTSB*, *CTSS*) in all subtypes whereas genes mediating resistance were not, except for *NANS* (sialic acid synthase) and *HSP90AB1* (heat shock protein 90 alpha family class B member 1). These data suggest that breast cancers may potentially benefit from several ADCs currently under development.

3.8. Integrated view of target expression and sensitivity and resistance markers

We conducted an exploratory analysis to sub-select ADC targets with the highest differential expression in each cancer type (Fig. 6A, Supplementary Table 10).

We highlight that there is substantial heterogeneity across tumours in overexpression of ADC targets. Since multiple tumour intrinsic factors contribute to determining response or resistance to a particular ADC in a given cancer, we attempted to synthesise these into a single-sample "*targetgram*" that shows the relative expression levels of different ADC targets along with the average expressions of resistance and sensitivity markers (Fig. 6B). Within each cancer type, heterogeneous expression profiles were observed. We also created a free web tool that can generate *targetgrams* with the mean expression of samples in any selected tissue type using multiple current or future targets as input.

4. Discussion

By exploiting large publicly available transcriptomic datasets, we performed a comprehensive mapping of molecules that are implicated in ADC activity in a broad range of solid tumours and normal tissues. Our results extend an earlier effort that evaluated ADC target expression across cancer types [22]. We assessed target expression simultaneously with genes involved in receptor internalisation, lysosomal trafficking, linker lysis, and payload sensitivity in both normal and malignant tissues, and examined expression levels in primary tumours as well as in metastatic lesions. We showed that for most ADC targets the mRNA expression levels correlate with protein expression, and

therefore large transcriptional profile databases can be used to estimate target expression at the protein level. We chose to measure ADC target expression levels relative to pooled normal tissues that provided a common reference to compare relative expression across and within tumour types. As a result, we identified for each target the most appealing cancer types for the corresponding ADC development. Our method confirmed high expression of ADC target in the cancer types for which ADCs are already approved (e.g. breast cancer and anti-HER2 and anti-Trop2 ADCs, ovarian cancer and anti-FOLR1 ADC, bladder cancer and anti-NECTIN4 and anti-Trop2 ADCs [3,6,10]), which supports the validity of this approach in finding novel areas of therapeutic investigation. More importantly, we identified numerous therapeutic opportunities suggested by high target expression in cancer types that are not currently studied in clinical trials (e.g. testicular cancers may be sensitive to anti-claudin-6 ADCs, uterine carcinosarcoma to anti-PTK7 ADCs etc.). We also document the co-expression of multiple different ADC targets in cancer, which opens opportunities to design future clinical trials with ADC combinations (e.g. anti-NECTIN4 plus anti-Trop2 in lung, breast, ovarian cancers, or anti-HER2 plus anti-*ERBB3* in lung, breast, and prostate cancers), or develop bispecific ADCs.

Response to ADCs is influenced by many biological processes other than target expression levels. We observed large variations in the relative expression levels of genes possibly implicated in response or resistance downstream of the target. These expression distributions can add additional granularity to the estimate of the fraction of tumours that might benefit from a given ADC in a particular tumour type. We attempted to synthesise the various components of ADC activity into a *targetgram* that shows the relative expression levels of different ADC targets and the average expressions of resistance and sensitivity markers in single samples. This could be extremely useful considering the high inter-patient heterogeneity within each histology and the evolving clinical landscape with multiple competitive ADC options available to be selected and properly sequenced.

ADCs have substantial toxicities despite extensive efforts to select target antigens with large differential expression between cancer and normal tissues. ADC-related adverse events may be caused by on-target but off-cancer effects due to shared antigen expression between normal and cancer tissues, or due to free cytotoxic payload released into the circulation. We evaluated the association between target expression data and expression of putative sensitivity and resistance markers on normal tissues with observed ADC-related adverse events. Target expression in normal tissues only partly and imprecisely explained toxicities observed in clinical trials. High expression of determinants of payload activity in healthy tissues appeared to explain more of the observed toxicities including haematological and lung toxicities of SG and T-DXd that could be due to high *TOP1* expression in the bone marrow and lung.

This study has several limitations. First, although correlations between mRNA and protein levels were high for most targets, the level of protein expression that is required for ADC activity is not established for any current ADC. This threshold likely varies by ADC design and tissue type. For some proteins such as ERBB3, the correlation between mRNA and protein is poor, so results need cautious interpretation. Furthermore, we used mRNA expressions from bulk RNA sequencing, and the precise tumour cell contributions to the RNA signal are not known. This may particularly affect genes involved in ADC response and resistance that represent cellular processes present in many normal cell types. We also recognise that many molecular determinants of sensitivity to an ADC and its payload remain unknown and the list of genes implicated in treatment response will likely increase rapidly in the coming years along with the repertoire of clinically relevant ADC targets. We also did not consider the currently poorly understood potential interactions between ADCs and the tumour microenvironment, including immune infiltration, that could also affect treatment response. Lastly, despite repeated searches with different terms, the rapidly evolving ADC scenario could not allow us to capture all clinical trials (e.g. the STATICE/NCCH1615 trial [23], testing trastuzumab deruxtecan in uterine carcinosarcoma, which was not present on clinicaltrials.gov, or NCT04154956 testing SAR408701 in non-squamous non-small-cell lung cancer, which was missed with used search terms).

5. Conclusion

Despite some constraints, our analysis is the most comprehensive assessment of the therapeutic potential of ADCs across a broad range of cancer types using currently available data. Through the investigation of large publicly available datasets, we identify novel therapeutic opportunities for ADCs that can be tested in the clinic. Our web-interactive tools can assist in prioritising novel emerging ADC targets for clinical development.

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Research data for this article

This work is based on the analysis of publicly available datasets and did not require generation of new data. Input data and scripts used to generate the results are available at https://github.com/mdugo/pan-cancer_ADC.

CRediT authorship contribution statement

Carlo Bosi: Data curation, Investigation, Methodology, Writing – original draft. **Áron Bartha**: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. **Barbara Galbardi**: Data curation, Methodology, Formal analysis, Investigation, Visualization, Writing – review & editing. **Giulia Notini**: Data curation, Investigation, Writing – review & editing. **Matteo M. Naldini**: Writing – review & editing. **Luca Licata**: Data curation, Visualization, Writing – review & editing. **Giulia Viale**: Writing – review & editing. **Marco Mariani**: Visualization, Writing – review & editing. **Barbara Pistilli**: Writing – review & editing. **H. Raza Ali**: Writing – review & editing. **Fabrice André:** Writing – review & editing. **Marta Piras**: Data curation, Writing – Review & editing. **Maurizio Callari:** Methodology, Visualization, Investigation, Writing – review & editing. **Marco Barreca**: Methodology, Writing – review & editing. **Alberta Locatelli:** Methodology, writing – review & editing. **Lucia Viganò**: Methodology, writing – review & editing. **Carmen Criscitiello:** Writing – review & editing. **Lajos Pusztai**: Supervision, Methodology, Writing – original draft. **Giuseppe Curigliano**: Writing – review & editing. **Balazs Győrffy**: Methodology, Software, Resources, Investigation, Supervision, Project administration, Writing – review & editing. **Matteo Dugo**: Methodology, Formal Analysis, Investigation, Visualization, Supervision, Project administration, Writing – review & editing. **Giampaolo Bianchini**: Conceptualization, Funding acquisition, Investigation, Methodology, Visualization, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

C.B.: Financial interests: personal consulting fees from Kardo srl (personal); travel support for attending meetings from Daiichi-Sankyo and Lilly.

G.N.: Travel support for attending meetings from Lilly and Sanofi.

M.M.: Travel support for attending meetings from Lilly.

L.L.: Financial interests: Consulting fees from Exact Sciences, Helsinn, EISAI; honoraria for speakers' bureaus from Gilead, Exact Sciences, Helsinn; support for attending meetings from Lilly and Gilead; advisory board for Lilly, Exact Sciences, AstraZeneca, Italfarmaco, Accord, Seagen and Daiichi Sankyo (all personal and financial).

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C.C.: Financial interests: Personal fees for consulting, advisory role, and speakers' bureau from Lilly, Roche, Novartis, MSD, Seagen, Gilead, Daiichi Sankyo, AstraZeneca, and Pfizer.

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A.B., B.Ga, H.R.A., M.C., M.B., A.L., L.V., M.D., M.M.N., and B.G. declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2023.113379.

References

[1] Modi S, Saura C, Yamashita T, Park YH, Kim S-B, Tamura K, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. N Engl J Med 2020;382:610–21. https://doi.org/10.1056/nejmoa1914510.

[2] Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med 2022;387:9–20. https://doi.org/10.1056/nejmoa2203690.

[3] Meric-Bernstam F, Makker V, Oaknin A, Oh D-Y, Banerjee SN, Gonzalez Martin A, et al. Efficacy and safety of trastuzumab deruxtecan (T-DXd) in patients (pts) with HER2-expressing solid tumors: DESTINY-PanTumor02 (DP-02) interim results. J Clin Oncol 2023;41:LBA3000. https://doi.org/10.1200/jco.2023.41.17_suppl.lba3000.

[4] Mosele F, Deluche E, Lusque A, Le Bescond L, Filleron T, Pradat Y, et al. Trastuzumab deruxtecan in metastatic breast cancer with variable HER2 expression: the phase 2 DAISY trial. Nat Med 2023;29:2110–20. https://doi.org/10.1038/s41591-023-02478-2.

[5] Colombo R, Rich JR. The therapeutic window of antibody drug conjugates: a dogma in need of revision. Cancer Cell 2022;40:1255–63. https://doi.org/10.1016/j.ccell.2022.09.016.

[6] Matulonis UA, Lorusso D, Oaknin A, Pignata S, Dean A, Denys H, et al. Efficacy and safety of mirvetuximab soravtansine in patients with platinum-resistant ovarian cancer with high folate receptor alpha expression: results from the SORAYA study. J Clin Oncol 2023;41:2436–45. https://doi.org/10.1200/JCO.22.01900.

[7] Moore KN, Oza AM, Colombo N, Oaknin A, Scambia G, Lorusso D, et al. Phase III, randomized trial of mirvetuximab soravtansine versus chemotherapy in patients with platinum-resistant ovarian cancer: primary analysis of FORWARD I. Ann Oncol 2021;32:757–65. https://doi.org/10.1016/j.annonc.2021.02.017.

[8] Hassan R, Blumenschein GR, Moore KN, Santin AD, Kindler HL, Nemunaitis JJ, et al. First-in-human, multicenter, phase i dose-escalation and expansion study of anti-mesothelin antibody-drug conjugate anetumab ravtansine in advanced or metastatic solid tumors. J Clin Oncol 2020;38:1824–35. https://doi.org/10.1200/JCO.19.02085.

[9] Strickler JH, Weekes CD, Nemunaitis J, Ramanathan RK, Heist RS, Morgensztern D, et al. First-in-human phase I, dose-escalation and -expansion study of telisotuzumab vedotin, an antibody–drug conjugate targeting c-Met, in patients with advanced solid tumors. J Clin Oncol 2018;36:3298–306. https://doi.org/10.1200/JCO.2018.78.7697.

[10] Klümper N, Ralser DJ, Ellinger J, Roghmann F, Albrecht J, Below E, et al. Membranous NECTIN-4 expression frequently decreases during metastatic spread of urothelial carcinoma and is associated

with enfortumab vedotin resistance. Clin Cancer Res 2023;29:1496–505. https://doi.org/10.1158/1078-0432.CCR-22-1764.

[11] Bardia A, Tolaney SM, Punie K, Loirat D, Oliveira M, Kalinsky K, et al. Biomarker analyses in the phase III ASCENT study of sacituzumab govitecan versus chemotherapy in patients with metastatic triple-negative breast cancer. Ann Oncol 2021;32:1148–56. https://doi.org/10.1016/j.annonc.2021.06.002.

[12] Rugo HS, Bardia A, Marmé F, Cortés J, Schmid P, Loirat D, et al. Overall survival with sacituzumab govitecan in hormone receptor-positive and human epidermal growth factor receptor 2-negative metastatic breast cancer (TROPiCS-02): a randomised, open-label, multicentre, phase 3 trial. Lancet 2023;6736:1–11. https://doi.org/10.1016/s0140-6736(23)01245-x.

[13] Kimberly Tsui C, Barfield RM, Fischer CR, Morgens DW, Li A, H Smith BA, et al. CRISPR-Cas9 screens identify regulators of antibody–drug conjugate toxicity. Nat Chem Biol 2019;15:949–58. https://doi.org/10.1038/s41589-019-0342-2.

[14] Wilks C, Zheng SC, Chen FY, Charles R, Solomon B, Ling JP, et al. recount3: summaries and queries for large-scale RNA-seq expression and splicing. Genome Biol 2021;22:1–40. https://doi.org/10.1186/s13059-021-02533-6.

[15] Jiang L, Wang M, Lin S, Jian R, Li X, Chan J, et al. A quantitative proteome map of the human body. Cell 2020;183:269–283.e19. https://doi.org/10.1016/j.cell.2020.08.036.

[16] Bartha Á, Győrffy B. TNMplot.com: a web tool for the comparison of gene expression in normal, tumor and metastatic tissues. Int J Mol Sci 2021;22:1–12. https://doi.org/10.3390/ijms22052622.

[17] Caculitan G, Chuh C, Ma Y, Zhang D, Kozak KR, Liu Y, et al. Cathepsin B is dispensable for cellular processing of cathepsin B-cleavable antibody – drug conjugates. Cancer Res 2017;77(24):7027–37. https://doi.org/10.1158/0008-5472.CAN-17-2391.

[18] Burgess DJ, Doles J, Zender L, Xue W, Ma B, McCombie WR, et al. Topoisomerase levels determine chemotherapy response in vitro and in vivo. Proc Natl Acad Sci U S A 2008;105:9053–8. https://doi.org/10.1073/pnas.0803513105.

[19] Hunter FW, Barker HR, Lipert B, Rothé F, Gebhart G, Piccart-Gebhart MJ, et al. Mechanisms of resistance to trastuzumab emtansine (T-DM1) in HER2-positive breast cancer. Br J Cancer 2020;122:603–12. https://doi.org/10.1038/s41416-019-0635-y.

[20] Chen R, Herrera AF, Hou J, Chen L, Wu J, Guo Y, et al. Inhibition of MDR1 overcomes resistance to brentuximab vedotin in Hodgkin lymphoma. Clin Cancer Res 2020;26:1034–44. https://doi.org/10.1158/1078-0432.CCR-19-1768.

[21] Tarantino P, Ricciuti B, Pradhan SM, Tolaney SM. Optimizing the safety of antibody–drug conjugates for patients with solid tumours. Nat Rev Clin Oncol 2023;20:558–76. https://doi.org/10.1038/s41571-023-00783-w.

[22] Moek KL, de Groot DJA, de Vries EGE, Fehrmann RSN. The antibody-drug conjugate target landscape across a broad range of tumour types. Ann Oncol 2017;28:3083–91. https://doi.org/10.1093/annonc/mdx541.

[23] Nishikawa T, Hasegawa K, Matsumoto K, Mori M, Hirashima Y, Takehara K, et al. Trastuzumab Deruxtecan for Human Epidermal Growth Factor Receptor 2–Expressing Advanced or Recurrent Uterine Carcinosarcoma (NCCH1615): The STATICE Trial. J Clin Oncol 2023;41:2789–99. https://doi.org/10.1200/JCO.22.02558.