The antibody-drug conjugate target landscape across a broad range of tumour types

Moek, K. L.; de Groot, D. J. A.; de Vries, E. G. E.; Fehrmann, R. S. N.

Published in:
Annals of Oncology

DOI:
10.1093/annonc/mdx541

Publication date:
2017

ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

Annals of Oncology 28: 3083–3091, 2017
doi:10.1093/annonc/mdx541
Published online 25 September 2017

ORIGINAL ARTICLE

The antibody–drug conjugate target landscape across a broad range of tumour types

K. L. Moek, D. J. A. de Groot, E. G. E. de Vries & R. S. N. Fehrmann*

Department of Medical Oncology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

*Correspondence to: Dr Rudolf S. N. Fehrmann, Department of Medical Oncology, University Medical Centre Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands. Tel: +31-503612821; Fax: +31-503614862; E-mail: r.s.n.fehrmann@umcg.nl

Background: Antibody–drug conjugates (ADCs), consisting of an antibody designed against a specific target at the cell membrane linked with a cytotoxic agent, are an emerging class of therapeutics. Because ADC tumour cell targets do not have to be drivers of tumour growth, ADCs are potentially relevant for a wide range of tumours currently lacking clear oncogenic drivers. Therefore, we aimed to define the landscape of ADC targets in a broad range of tumours.

Materials and methods: PubMed and ClinicalTrials.gov were searched for ADCs that are or were evaluated in clinical trials. Gene expression profiles of 18 055 patient-derived tumour samples representing 60 tumour (sub)types and 3520 healthy tissue samples were collected from the public domain. Next, we applied Functional Genomic mRNA-profiling to predict per tumour type the overexpression rate at the protein level of ADC targets with healthy tissue samples as a reference.

Results: We identified 87 ADCs directed against 59 unique targets. A predicted overexpression rate of $\geq$ 10% of samples for multiple ADC targets was observed for high-incidence tumour types like breast cancer ($n = 31$ with $n = 23$ in triple negative breast cancer), colorectal cancer ($n = 18$), lung adenocarcinoma ($n = 18$), squamous cell lung cancer ($n = 16$) and prostate cancer ($n = 5$). In rare tumour types we observed, amongst others, a predicted overexpression rate of 55% of samples for CD22 and 55% for ENPP3 in adrenocortical carcinomas, 81% for CD74 and 81% for FGFR3 in osteosarcomas, and 95% for c-MET in uveal melanomas.

Conclusion: This study provides a data-driven prioritization of clinically available ADCs directed against 59 unique targets across 60 tumour (sub)types. This comprehensive ADC target landscape can guide clinicians and drug developers which ADC is of potential interest for further evaluation in which tumour (sub)type.

Key words: antibody–drug conjugate, FGmRNA-profiling, target, cancer

Introduction

Despite progress in anticancer drug treatment including molecularly targeted agents that inhibit specific oncogenic 'driver' pathways, most patients still die of metastatic disease. Therefore, there remains an unmet need to develop new systemic treatment options to improve survival of cancer patients.

Numerous patients fail to benefit from molecularly targeted agents because their tumours lack oncogenic drivers to target. In this context, an interesting emerging class of therapeutics are antibodies bound to a cytotoxic agent, known as antibody–drug conjugates (ADCs). ADC targets do not have to be drivers of tumour growth to be meaningful because they serve as an entry point for the cytotoxic agent. This makes ADCs potentially relevant for a wide range of tumours.

After an ADC is bound to its tumour-specific molecular target, the cytotoxin is internalized and activated. This allows the selective cellular tumour delivery of a high concentration of the cytotoxin that would cause severe dose-limiting toxicities if administered systemically. To prevent unintended biodistribution, the total body target expression should favour the tumour instead of healthy tissues [1]. An established example of an ADC is trastuzumab emtansine, which is currently part of standard of care in patients with human epidermal growth factor receptor 2 (HER2) overexpressing metastatic breast cancer [2].

Immunohistochemical (IHC) analyses allows to investigate the protein expression of ADC targets in different tumour (sub)types and healthy tissues. However, large-scale IHC analysis for a target is time-consuming and demands extensive resources. Therefore, we currently lack data about the expression of ADC targets for numerous tumour types, which impedes potential effective treatment with available ADCs in a significant subset of cancer patients.

To this end, we used the recently developed method of functional genomic mRNA profiling (FGmRNA-profiling) to predict overexpression rates of ADC targets at the protein level [3]. FGmRNA-profiling can correct a gene expression profile of an individual tumour for physiological and experimental factors, which are considered not to be relevant for the observed tumour phenotype.

In this article, we applied FGmRNA-profiling to a large database containing a broad spectrum of different tumour and healthy tissue (sub)types. Subsequently, we used the resulting FGmRNA-profiles to prioritize potential ADC targets per tumour (sub)type. In addition, we present an overview of ADCs that are currently marketed or in clinical development for anticancer treatment.

Materials and methods

Search strategy

To identify targets for clinically available ADCs, PubMed was searched at the latest April 2017. The following search terms were used: 'antibody-drug conjugate', 'cancer', 'tumour' and 'oncology' in various combinations, spelling variants and synonyms. The search was limited to manuscripts published in English and involving clinical trials. Reviews were excluded. In addition, ClinicalTrials.gov was searched in April 2017 for ongoing studies with ADCs with the search terms [antibody-drug conjugate] AND [cancer]. Finally, abstracts and posters from the ASCO 2015/2016 and ECCO-ESMO 2015 and ESMO 2016 meetings were selected using 'antibody-drug conjugate' as search term.

Moreover, information on ADCs, ADC targets, linked cytotoxins, tumour type and status of clinical development (phase I–III) was collected. If we could not find that information in the previously described sources, we searched Embase to collect additional information using the name of the identified ADC as term. In case an ADC is in different phases of clinical development for a specific indication, we chose to systematically report the highest phase.

Data acquisition

Publicly available microarray expression data were extracted from the Gene Expression Omnibus (GEO) [4]. The analysis was confined to the Affymetrix HG-U133 Plus 2.0 platform (GEO accession identifier: GPL570). Samples were included for analysis if they represented healthy tissue or cancer tissue obtained from patients or healthy individuals and raw data was available. Only tumour (sub)types with ≥ 5 samples were included for analysis. Preprocessing and quality control was carried out as previously described [3, 5]. For the breast cancer, cohort receptor status was collected or inferred as described before [5, 6].

Predicting overexpression rates of ADC targets at the protein level

First, we applied FGmRNA-profiling to each individual sample, both cancer and healthy tissue. For a detailed description of FGmRNA-profiling, we refer to Fehrmann et al. [3]. In short, we analysed 77 840 expression profiles of publicly available samples with principal component analysis and found that a limited number of 'Transcriptional Components' (TCs) capture the major regulators of the mRNA transcriptome. Subsequently, we identified a subset of TCs that described non-genetic regulatory factors. We used these non-genetic TCs as covariates to correct microarray expression data and observed that the residual expression signal (i.e. FGmRNA-profile) captures the downstream consequences of genomic alterations on gene expression levels.

Subsequently, for each individual gene that is targeted by ADC(s), we determined the percentage of samples per tumour (sub)type with a significant increased FGmRNA-signal, which is considered a proxy for protein overexpression. The threshold was defined in the set of FGmRNA-profiles of healthy tissues by calculating the 97.5th percentile for the FGmRNA-signal of the target under investigation. For each individual tumour sample, the gene under investigation was marked as overexpressed when the FGmRNA-signal was above the 97.5th percentile threshold as defined in the healthy tissue samples. Per tumour (sub)type, the percentage of samples with marked overexpression is reported per target. In addition, we determined the number of ADC targets showing predicted overexpression in $\geq 75\%$, $\geq 50\%$ and $\geq 25\%$ of samples for at least one tumour type. As the Affymetrix HG-U133 Plus 2.0 platform contains multiple probes representing an individual gene, we choose to systematically report per tumour (sub)type the probe with the highest predicted percentage of samples with a significant increased FGmRNA-signal.

In addition, we predicted ADC target overexpression based on regular mRNA data by applying the same methodology as described above.

Results

Identified ADCs

A total of 87 ADCs were identified of which two are registered for use in humans and 55 are currently under clinical evaluation (Table 1 and supplementary Table S1, available at *Annals of Oncology* online). For 16 ADCs, clinical evaluation was terminated for various reasons and the status of clinical evaluation of 14 ADCs is unknown. In total, 61 ADCs are studied in solid tumours, 21 in haematological malignancies and 5 in both solid and haematological malignancies. In solid tumours, the largest number of ADCs ($n = 24$) is evaluated in breast cancer including 12 in triple negative breast cancer (TNBC), followed by non-small-cell lung cancer (NSCLC) ($n = 18$), gastric cancer ($n = 16$) and ovarian cancer ($n = 16$) (Figure 1). Supplementary Figures S1 and S2, available at *Annals of Oncology* online, provide a comprehensive overview of ADCs in clinical development for the treatment of solid, haematological and paediatric tumours.

Eight ADCs are currently in phase III trials, including the registered brentuximab vedotin and trastuzumab emtansine. Twenty-two ADCs are evaluated in phase II trials and 7 ADCs that have been evaluated in phase II trials did not proceed to phase III for various reasons. In addition, 28 ADCs are tested in phase I clinical trials and 22 have been assessed but did not (yet) proceed to phase II for several reasons. Detailed information can be found in Table 1 and supplementary Figures S1 and S2, available at *Annals of Oncology* online.

Identified ADCs targets

Targets are publicly disclosed for 84 of the 87 ADCs (Table 1). These 84 ADCs target 59 unique targets. Eight ADCs are directed against HER2, including trastuzumab emtansine. In addition, the

Table 1. Overview of registered ADCs and ADCs in clinical trials for cancer treatment

Target	Cytotoxin	ADC	Phase
5T4	MMAF	PF-06263507[a]	1
AXL	MMAE	HuMax-AXL-ADC	2
BCMA	MMAF	GSK2857916	1
c-MET	MMAE	ABBV-399	1
C4.4a	Auristatin W derivative	BAY1129980	1
CA6	DM4	SAR566658	2
CA9	MMAE	BAY79-4620[b]	1
Cadherin-6	Maytansine	HKT288	1
CD19	DM4	Coltuximab ravtansine[c]	2
CD19	MMAF	Denintuzumab mafodotin	2
CD19	PBD	ADCT-402	1
CD19	PBD	SGN-CD19B	1
CD22	Calicheamicin	Inotuzumab ozogamicin	3
CD22	MMAE	Pinatuzumab vedotin	2
CD25	PBD	ADCT-301	1
CD27L	DM1	AMG 172[d]	1
CD30	MMAE	Brentuximab vedotin	Registered
CD33	Calicheamicin	Gemtuzumab ozogamicin	3
CD33	DM4	AVE9633[e]	1
CD33	PBD	Vadastuximab talirine	3
CD37	DM4	IMGN529	2
CD37	MMAE	AGS67E	1
CD44v6	DM1	Bivatuzumab mertansine[b]	1
CD56	DM1	Lorvotuzumab mertansine[f]	2
CD70	Duocarmycin	MDX-1203[d]	1
CD70	MMAE	Vorsetuzumab mafodotin[d]	1
CD70	MMAF	SGN-CD70A	1
CD74	Doxorubicin	Milatuzumab doxorubicin[c]	2
CD79b	MMAE	Polatuzumab vedotin[g]	2
CD123	PBD	SGN-CD123A	1
CD138	DM4	Indatuximab ravtansine	2
CEA	DM4	SAR408701	2
CEA	SN-38	Labetuzumab govitecan	2
cKit	Maytansine	LOP628[h]	1
Cripto protein	DM4	BIIB015[d]	1
CS1	MMAE	ABBV-838	1
DLL3	Not disclosed	SC-002	1
DLL3	PBD	Rovalpituzumab tesirine	3
EDNRB	MMAE	DEDN6526A[e]	1
EFNA4	Calicheamicin	PF-06647263	1
EGFR	DM1	IMGN289[b]	1
EGFR	MMAF	ABT-414	2
EGFRvIII	DM1	AMG 595[d]	1
ENPP3	MMAF	AGS-16C3F	2
EPHA2	MMAF	MEDI-547[b]	1
FGFR2	Auristatin W derivative	BAY1187982[e]	1
FGFR3	DM4	LY3076226	1
FLT3	Not disclosed	AGS62P1	1
FOLR1	DM4	Mirvetuximab soravtansine	3
GPNMB	MMAE	Glembatumumab vedotin	2
GUCY2C	MMAE	MLN0264	2
HER2	Auristatin payload	XMT-1522	1
HER2	DM1	Trastuzumab emtansine	Registered
HER2	Duocarmycin	SYD-985	1
HER2	DXd	DS-8201A	1
HER2	Liposomal doxorubicin	MM-302[i]	2

Continued

Table 1. *Continued*

Target	Cytotoxin	ADC	Phase
HER2	MMAE	RC48-ADC	2
HER2	MMAF	ARX788	1
HER2	Tubulysin	MEDI-4276	2
HER3	DXd	U3-1402	2
Integrin alpha	DM4	IMGN388[a]	2
LAMP-1	DM4	SAR428926	1
Lewis Y	Doxorubicin	SGN-15[a]	2
LIV-1	MMAE	SGN-LIV1A	1
LRRC15	MMAE	ABBV-085	1
MSLN	DM4	Anetumab ravtansine	2
MSLN	MMAE	DMOT4039A[e]	1
MSLN	Not disclosed	BMS-986148	2
MUC1	DM1	Cantuzumab mertansine[j]	1
MUC1	DM4	Cantuzumab ravtansine[a]	2
MUC16	MMAE	Sofituzumab vedotin[d]	1
NaPi2b	MMAE	Lifastuzumab vedotin	2
Nectin-4	MMAE	Enfortumab vedotin	1
NOTCH3	Auristatin payload	PF-06650808[d]	1
p-CAD	Not disclosed	PCA-062	1
PSMA	DM1	MLN2704[e]	2
PSMA	MMAE	PSMA ADC 1301[c]	2
PTK7	Auristatin	PF-06647020	1
SLC44A4	MMAE	ASG-5ME[k]	1
SLITRK6	MMAE	ASG-15ME[d]	1
STEAP1	MMAE	Vandortuzumab vedotin[d]	1
TF	MMAE	Tisotumab vedotin	2
TIM-1	MMAE	CDX-014	1
TROP-2	SN-38	Sacituzumab govitecan	3
Not disclosed	MMAE	DFRF4539A[d]	1
Not disclosed	Not disclosed	AbGn-107	1
Not disclosed	Not disclosed	SC-003	1

[a]Development discontinued to focus on other product candidates.
[b]Development terminated due to safety reasons.
[c]According to ClinicalTrials.gov, no ongoing phase II studies on 16 February 2017. Development status: unknown.
[d]According to ClinicalTrials.gov, no ongoing phase I studies on 16 February 2017. Development status: unknown.
[e]Development discontinued (not further specified).
[f]Phase II study stopped prematurely due to no significant benefit and possible harm in SCLC. Phase II studies in leukaemia and paediatric tumours still ongoing.
[g]Development has been discontinued in CLL after phase I evaluation, development on-going in NHL.
[h]Phase I study terminated prematurely.
[i]Phase II/III study terminated because it failed to show benefit over control arm per DMC and confirmed via futility analyses.
[j]Development terminated due to the company's decision to replace DM1 with DM4.
[k]Development discontinued in gastric and pancreatic cancer, unknown status in prostate cancer.
AXL, AXL receptor tyrosine kinase; BCMA, B-cell maturation antigen; CA6, carbonic anhydrase 6; CA9, carbonic anhydrase 9; CD, cluster of differentiation; CEA, carcinoembryonic antigen; CLL, chronic lymphocytic leukaemia; DLL3, delta-like canonical Notch ligand 3; DMC, data monitoring committee; EDNRB, endothelin receptor type B; EFNA4, ephrin A4; EGFR, epidermal growth factor receptor; ENPP3, ectonucleotide pyrophosphatase/phosphodiesterase 3; EPHA2, EPH receptor A2; FGFR2, fibroblast growth factor receptor 2; FGFR3, fibroblast growth factor receptor 3; FLT3, FMS-like tyrosine kinase 3; FOLR1, folate receptor 1; GPNMB, glycoprotein non-metastatic B; GUCY2C, guanylate cyclase 2 C; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; LAMP-1, lysosomal-associated membrane protein 1; LRRC15, leucine rich repeat containing 15; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; MSLN, mesothelin; MUC1, mucin 1; MUC16, mucin 16; NaPi2b, sodium-dependent phosphate transport protein 2B; NHL, non-Hodgkin lymphoma; NOTCH3, notch 3; p-CAD, p-cadherin; PBD, pyrrolobenzodiazepine; PSMA, prostate-specific membrane antigen; PTK7, protein tyrosine kinase 7; SLC44A4, solute carrier family 44 member 4; SCLC, small-cell lung cancer; SLITRK6, SLIT like family member 6; STEAP1, STEAP family member 1; TF, tissue factor; TIM-1, T cell immunoglobulin and mucin protein-1; TROP-2, trophoblast cell-surface antigen.

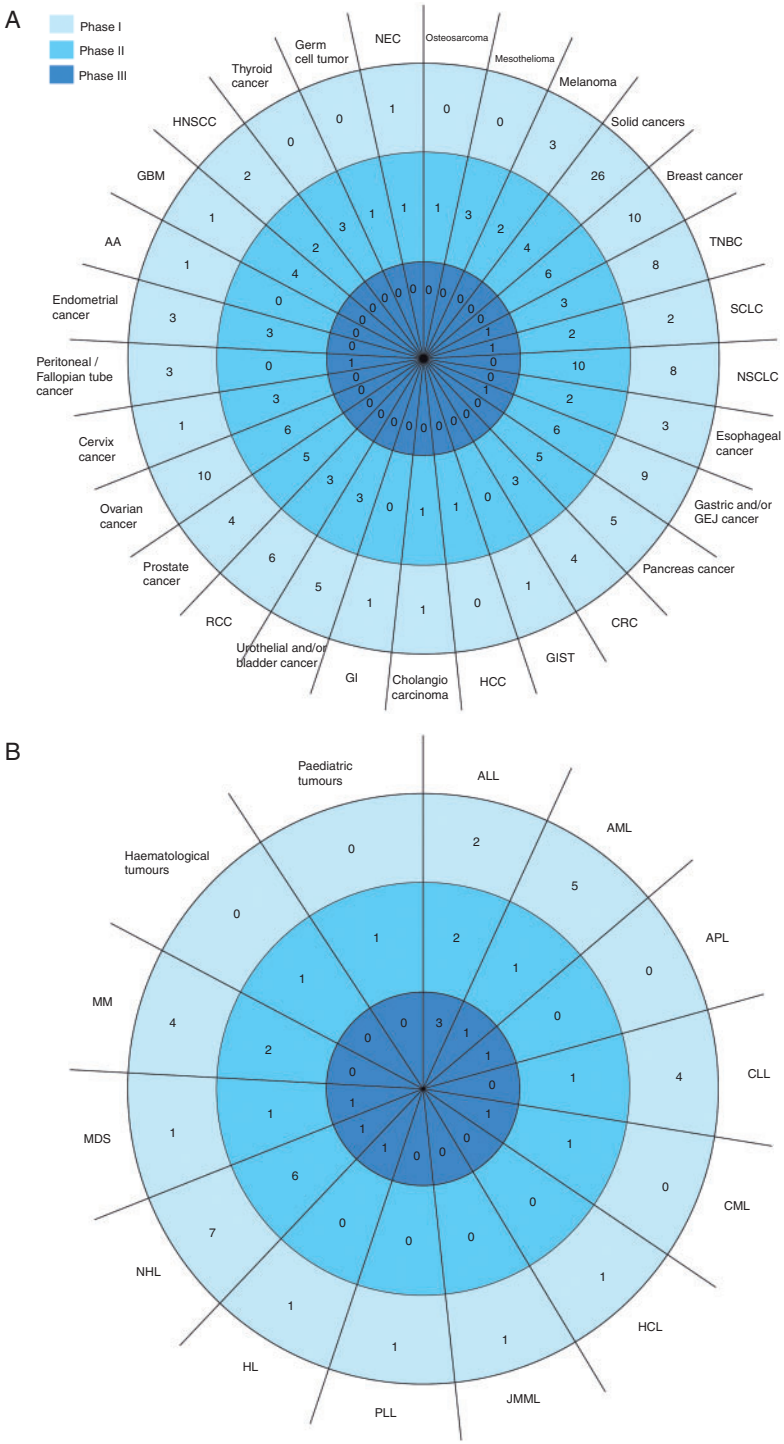

Figure 1. ADCs in clinical trials for treatment of solid tumours (A) and haematological and paediatric tumours (B). The total number of identified ADCs under clinical evaluation is shown per tumour type and per stage of clinical development. More extensive information can be found in supplementary Figures S1 and S2, available at *Annals of Oncology* online. The US Food and Drug Administration (FDA) and European Medicines Agency (EMA) registered trastuzumab emtansine for the treatment of HER2-positive metastatic breast cancer and brentuximab vedotin for treatment of NHL are not shown. AA, anaplastic astrocytomas; ADC, antibody–drug conjugate; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; APL, acute promyelocytic leukaemia; cholangio, cholangio carcinoma; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; CRC, colorectal cancer; GBM, glioblastoma multiforme; GEJ, gastro-oesophageal junction; GI, gastrointestinal; GIST, gastrointestinal stromal tumour; HCC, hepatocellular carcinoma; HCL, hairy cell leukaemia; HER2, human epidermal growth factor receptor 2; HL, Hodgkin lymphoma; HNSCC, head and neck squamous cell carcinoma; JMML, juvenile myelomonocytic leukaemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; NEC, neuroendocrine carcinoma; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; PLL, prolymphocytic leukaemia; RCC, renal cell cancer; SCLC, small-cell lung cancer; TNBC, triple negative breast cancer.

Table 2. Cytotoxins part of ADCs and their mechanism of action

Cytotoxin	Mechanism of action	Number of ADCs
Auristatin[a]	Inhibitor of tubulin polymerization	3
Auristatin W derivative	Inhibitor of tubulin polymerization	2
Calicheamicin	Causes DNA double strand breaks	3
DM1	Inhibitor of tubulin polymerization	8
DM4	Inhibitor of tubulin polymerization	13
Doxorubicin	Inhibitor of DNA relegation, causing DNA double strand breaks	2
Duocarmycin	Breaks down adenine-specific molecules in DNA	2
DXd	Topoisomerase inhibitor	2
Liposomal doxorubicin	Inhibitor of DNA relegation, causing DNA double strand breaks	1
Maytansine[a]	Inhibitor of tubulin polymerization	1
MMAE	Inhibitor of tubulin polymerization	26
MMAF	Inhibitor of tubulin polymerization	8
PBD	Interferes with the action of endonuclease enzymes on DNA and blocks transcription by inhibiting DNA polymerase in a sequence-specific manner	6
SN-38	Topoisomerase inhibitor	2
Tubulysin	Inhibitor of tubulin polymerization	1

[a]Not further specified.
MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; PBD, pyrrolobenzodiazepine; ADCs, Antibody–drug conjugates.

cluster of differentiation (CD) proteins CD19, CD33, CD70 and epidermal growth factor receptor (EGFR) and mesothelin (MSLN) are each targeted by at least three different ADCs.

Cytotoxins linked to ADCs

We identified 13 cytotoxins that are utilized in the set of 87 ADCs (Table 2). For six ADCs, the cytotoxin used is not publicly disclosed. The most frequently identified cytotoxins are the auristatins MMAE ($n = 26$) and MMAF ($n = 8$) and the maytansine derivatives DM4 ($n = 13$) and DM1 ($n = 8$). Detailed information is provided in Table 2.

Predicted protein overexpression rates of ADC targets by FGmRNA-profiling

We identified 18 055 samples representing 60 different tumour (sub)types and 3520 samples representing 22 healthy tissue (sub)types. We predicted protein overexpression rates for the 59 identified ADC targets. A predicted protein overexpression rate of $\geq 75\%$ of samples was observed for 17 ADC targets in at least one tumour (sub)type, $\geq 50\%$ for 38 and $\geq 25\%$ for 56. Figure 2 shows predicted protein overexpression rates for all 59 unique ADC targets in each of the 60 different tumour (sub)types. Detailed information can be found in supplementary Table S2, available at *Annals of Oncology* online.

Predicted overexpression for 59 unique ADC targets across 60 tumour (sub)types based on regular mRNA data is available as supplementary Table S3, available at *Annals of Oncology* online.

Predicted protein overexpression for ADC targets in frequently diagnosed tumour (sub)types. Predicted overexpression rate of $\geq 10\%$ of samples for multiple ADC targets was observed in colorectal cancer ($n = 18$), lung adenocarcinoma ($n = 18$), squamous cell lung cancer ($n = 16$) and prostate cancer ($n = 5$). Predicted overexpression rate of $\geq 10\%$ of samples was observed for 25 ADC targets in oestrogen receptor (ER)-negative/HER2-positive breast cancer, 23 in TNBC, 18 in ER-positive/HER2-positive breast cancer and 17 in ER-positive/HER2-negative breast cancer. Next, for the frequently occurring breast-, lung-, and prostate cancer, we highlight ADC targets with potential clinical impact as they have not been clinically explored in these tumour types.

For solute carrier family 44 member 4 (SLC44A4), a predicted overexpression rate of $\geq 35\%$ of samples was observed in all breast cancer subtypes except for only 9% in TNBC. In HER2-positive breast cancer, a predicted overexpression rate of 44% was observed for nectin-4 (PVRL4) and in ER-positive breast cancer 41% for fibroblast growth factor receptor 3 (FGFR3). In TNBC, the highest predicted overexpression rate with 51% was observed for nectin-4, followed by 39% for mucin 16 (MUC16). In lung adenocarcinomas we observed predicted overexpression rates of 36% for nectin-4 and 34% for ectonucleotide pyrophosphatase/phosphodiesterase 3 (ENPP3), whereas in squamous cell lung cancer 43% for carbonic anhydrase (CA9) and 42% for nectin-4. For protein tyrosine kinase 7 (PTK7) 11% predicted overexpression was found in prostate cancer.

Predicted protein overexpression for ADC targets in rare tumour (sub)types. We observed predicted overexpression of $\geq 10\%$ of samples for several ADC targets that have not been clinically explored in rare tumour types, with currently only limited treatment options, such as adrenocortical carcinomas, osteosarcomas, squamous cell oesophageal cancer and uveal melanomas (Figure 2). We observed a predicted overexpression rate of 55% of samples for

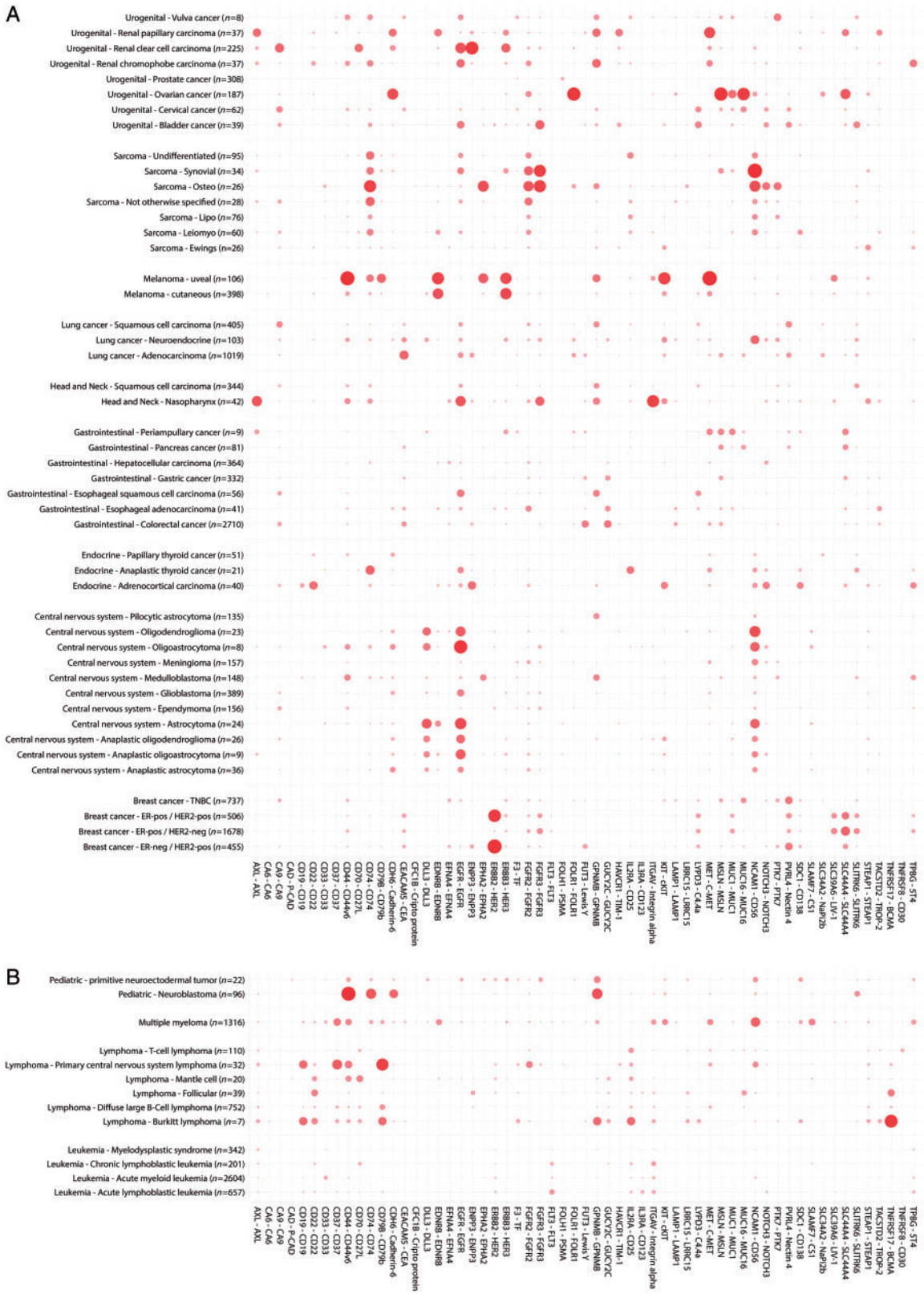

Figure 2. Predicted ADC target overexpression analysed by FGmRNA-profiling in solid tumours (A) and haematological and paediatric tumours (B). Predicted ADC target overexpression profiles per tumour type are represented as dots. The size of the dots indicates the percentage of patient-derived tumour samples with predicted overexpression of an ADC target, e.g. the larger the diameter, the more samples show target overexpression. The *x*-axis represents 59 identified ADC targets, both the gene and protein name are shown. ADC, antibody–drug conjugate; FGmRNA, functional genomic mRNA.

CD22 and 55% for ENPP3 in adrenocortical carcinomas, and 46% of samples for glycoprotein non-metastatic b (GPNMB) in squamous cell oesophageal cancer. In osteosarcomas, we show high predicted overexpression of FGFR3 and CD74 both in 81% of samples, followed by neural cell adhesion molecule 1 and ephrin type-A receptor 2 (EPHA2) (73%). In most other sarcoma subtypes tested, these ADC targets show similar overexpression patterns except for EPHA2. In uveal melanomas c-MET ranked the highest with an observed predicted overexpression rate of 95%, followed by CD44 (94%).

Predicted protein expression for ADC targets in healthy tissues. Detailed information concerning the distribution of individual ADC target mRNA-signals across 22 different healthy tissues is provided as supplementary Figure S3, available at *Annals of Oncology* online. For example, we observed relative high levels of mRNA-signals for GPNMB in a subset of healthy skin samples. In a phase II trial in 124 TNBC patients, being treated with the GPNMB-directed ADC glembatumumab vedotin, treatment-related skin rash was observed in 47% of patients, ranging from mild erythema to more involved maculopapular dermatologic toxicity [7]. In addition, treatment-related pruritus, hyperpigmentation and peeling were seen. Supplementary Table S4, available at *Annals of Oncology* online, shows per healthy tissue type the median ranked mRNA-signal for all ADC targets, which can be used to predict per healthy tissue type the ADC target with potential the highest toxicity. Supplementary Figure S4, available at *Annals of Oncology* online, shows the distribution of individual ADC target FGmRNA-signals across 22 different healthy tissues.

Predicted protein overexpression rates for all genes present in our dataset ($n = 22\,484$) for the 60 tumour (sub)types as determined with FGmRNA-profiling or regular mRNA-based analysis are provided in, respectively, supplementary Tables S5 and S6, respectively, available at *Annals of Oncology* online.

Discussion

A systematic search identified 87 ADCs directed against 59 unique targets that are or were evaluated in clinical trials for cancer treatment. Subsequently, we predicted protein overexpression rates for these 59 ADC targets in 60 tumour (sub)types utilizing FGmRNA-profiling.

FGmRNA-profiling is a recently developed method that is capable to correct a gene expression profile of an individual tumour for physiological and experimental factors, which are considered not to be relevant for the observed tumour phenotype [3]. We considered the residual mRNA levels (FGmRNA-signal) a better proxy for protein expression in tumour samples than regular mRNA expression levels. FGmRNA-profiling can only be applied to gene expression profiles generated with the Affymetrix HG-U133 Plus 2.0 platform, as this platform formed the basis for its development. The samples available for the Affymetrix HG-U133 Plus 2.0 platform still represent the most extensive collection of human gene expression profiling data available generated using a single uniform platform.

FGmRNA-profiling allows us to determine predicted protein overexpression rates for many potential druggable targets across a broad spectrum of tumour types in a rapid, efficient and consistent manner. In this article, we predicted 93% HER2 overexpression in histological proven ER-negative/HER2-positive and 85% in ER-positive/HER2-positive breast cancer, which serves as a positive validation of our methodology. Predicted overexpression of EGFR in NSCLC is ~30% lower than IHC data in literature; however, contrary to HER2 IHC testing, a standardized protocol for EGFR IHC analysis is lacking, which might have a strong impact on IHC results [8]. Moreover, we used FGmRNA-profiling to detect overexpression of *AXIN2*, *CEMIP*, *CD44* and *JUN* in expression profiles of colorectal adenomas when compared with a set of normal colon samples and confirmed these predictions in an independent set of colorectal adenomas with IHC analysis [9].

However, mRNA data must be interpreted with some caution; because mRNA transcripts might not always be translated to the protein, protein levels might be low due to high turnover or might not end up on the cell membrane [10]. In addition, expression profiles of complex biopsies obtained from tumours cannot inform us about tumour heterogeneity. Moreover, distinction between tumour cells and surrounding non-tumour cells as source of ADC target overexpression is difficult [6]. By using a large set of various healthy tissue samples as a reference to determine the threshold for 'overexpression', we could minimalize the effect of ADC target overexpression in non-tumour cells. However, IHC analyses have also some well-known disadvantages. Often highly heterogeneous scoring methods or different staining antibodies with varying antibody-target affinities are used, which impedes accurate comparison of IHC patterns in different studies of different tumour types. Also, it precludes a general cut-off for IHC indicating overexpression of the protein of interest. To illustrate this problem, we previously reported on 5 different anti-MSLN staining antibodies and 13 different scoring systems used in literature to study MSLN IHC expression in cancer, showing broad variation in MSLN-positivity, for example varying between 0% and 69% in NSCLC [6]. Therefore, obtaining a final estimated expression rate for a specific ADC target in a specific tumour type based on IHC results from literature is very hard and this hampers direct comparison with our predicted rates. However, the provided predicted expression rates in this article—which are approximations of expression rates obtained with IHC—have the advantage over IHC-based results that they are all obtained with exactly the same methodology. This allows researchers to directly compare the predicted expression rates between tumour (sub)types and target antigens to prioritize which ADC targets should be considered for subsequent recommended IHC validation and enables them to use resources more efficiently.

Design of effective ADCs requires appropriate target selection, which has proven to be surprisingly complex. ADC targets can be present either on tumour cells, tumour-associated cells (e.g. tumour endothelial cells) or in the tumour microenvironment [11]. Ideally the ADC target is highly overexpressed with limited heterogeneity at the cell membrane of tumour cells but is not, or only very limited, expressed at the cell membrane of healthy cells making the target (nearly) 'tumour-specific' [12]. However, most ADC targets are tumour-associated instead of tumour-specific and therefore the relative bio-distribution of ADCs to tumour and healthy tissue is often a limiting factor for broad clinical

applicability [1]. Extensive information about ADC target overexpression in healthy cells is not available in literature. Therefore, we also provided the mRNA-based expression levels for the 59 ADC targets in a set of 22 healthy tissue types, including organs at risk of toxicity such as liver, heart, and lung. Potentially, these data can be used to generate hypotheses regarding the potential toxicity of an ADC with a specific target.

In this study, we focussed on predicted protein overexpression rates of ADC targets in 60 tumour (sub)types. The ADC target landscape we created can also be applied to other antibody-related therapeutics, like bi-specific antibodies, immunotoxins (antibodies or antibody fragments fused with a toxin), radioimmunoconjugates (radiolabelled antibodies) and chimeric antigen receptors. In line with ADCs, these treatment approaches do not require a driver target to be successful.

In conclusion, our data provide clinicians and drug developers with an instrument that facilitates for further evaluation.

Funding

European Research Council advanced grant OnQview and the Dutch Cancer Society grant [RUG 2016-10034 (POINTING) to EGEdV and RUG 2013-5960 to RSNF]; the NWO-VENI grant (916-16025 to RSNF); a Mandema Stipendium (no grant number applicable to RSNF).

Disclosure

The authors have declared no conflicts of interest.

References

1. Tolcher AW. Antibody drug conjugates: lessons from 20 years of clinical experience. Ann Oncol 2016; 27: 2168–2172.
2. Verma S, Miles D, Gianni L et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012; 367: 1783–1791.
3. Fehrmann RS, Karjalainen JM, Krajewska M et al. Gene expression analysis identifies global gene dosage sensitivity in cancer. Nat Genet 2015; 47: 115–125.
4. Barrett T, Wilhite SE, Ledoux P et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic Acids Res 2013; 41: D991–D995.
5. Bense RD, Sotiriou C, Piccart-Gebhart MJ et al. Relevance of tumor-infiltrating immune cell composition and functionality for disease outcome in breast cancer. J Natl Cancer Inst 2017; 109: 1–9.
6. Lamberts LE, de Groot DJ, Bense RD et al. Functional genomic mRNA profiling of a large cancer data base demonstrates mesothelin overexpression in a broad range of tumor types. Oncotarget 2015; 6: 28164–28172.
7. Yardley DA, Weaver R, Melisko ME et al. EMERGE: a randomized phase II study of the antibody-drug conjugate glembatumumab vedotin in advanced glycoprotein NMB-expressing breast cancer. J Clin Oncol 2015; 33: 1609–1619.
8. Gaber R, Watermann I, Kugler C et al. Correlation of EGFR expression, gene copy number and clinicopathological status in NSCLC. Diagn Pathol 2014; 9: 165.
9. Hartmans E, Orian-Rousseau V, Matzke-Ogi A et al. Functional genomic mRNA profiling of colorectal adenomas: identification and *in vivo* validation of CD44 and splice variant CD44v6 as molecular imaging targets. Theranostics 2017; 7: 482–492.
10. Damelin M, Zhong W, Myers J, Sapra P. Evolving strategies for target selection for antibody-drug conjugates. Pharm Res 2015; 32: 3494–3507.
11. Thomas A, Teicher BA, Hassan R. Antibody-drug conjugates for cancer therapy. Lancet Oncol 2016; 17: e254–e262.
12. Beck A, Goetsch L, Dumontet C, Corvaïa N. Strategies and challenges for the next generation of antibody-drug conjugates. Nat Rev Drug Discov 2017; 16: 315–337.