onco

MDPI

Article

Adult B-Cell Acute Lymphoblastic Leukaemia Antigens and Enriched Pathways Identify New Targets for Therapy

Eithar Mohamed [1], Sara Goodman [1,†], Leah Cooksey [1,†], Daniel M. Fletcher [1], Olivia Dean [1], Viktoriya B. Boncheva [2], Ken I. Mills [3], Kim H. Orchard [4] and Barbara-ann Guinn [1,*]

[1] Centre for Biomedicine, Hull York Medical School, University of Hull, Cottingham Road, Kingston-Upon-Hull HU6 7RX, UK; e.a.mohamed-2019@hull.ac.uk (E.M.); sara1706@hotmail.co.uk (S.G.); l.cooksey@hull.ac.uk (L.C.); d.m.fletcher-2018@hull.ac.uk (D.M.F.)
[2] School of Life Sciences, University of Bedfordshire, Park Square, Luton LU1 3JU, UK; viktoriya.boncheva@outlook.com
[3] Patrick G. Johnson Centre for Cancer Research, Queen's University Belfast, Lisburn Road, Belfast BT9 7AE, UK; k.mills@qub.ac.uk
[4] Department of Haematology, University Hospital Southampton NHS Foundation Trust, Tremona Road, Southampton SO16 6YD, UK; kho@soton.ac.uk
* Correspondence: barbara.guinn@hyms.ac.uk
† These authors contributed equally to the study.

Simple Summary: Acute lymphoblastic leukaemia is a cancer of the immature white blood cells that reside in the bone marrow. It mostly affects children, with a peak incidence at 0–4 years, but 4 in 10 people affected by this type of leukaemia are adults. For adults with acute lymphoblastic leukaemia, the 5-year survival rates for those over 40 years of age is only 25%, and this disease desperately needs some new treatments to help improve the chances of survival. To this end, we have used a number of different methods to try to identify which proteins are altered in adult acute lymphoblastic leukaemia and which of the normal cell behaviours are affected by the disease process. Our study identified a number of proteins and cell behaviours that are affected in acute lymphoblastic leukaemia and these are worthy of further investigation as targets for new treatment strategies.

Abstract: Background: Adult B-cell acute lymphoblastic leukaemia (aB-ALL) is characterised by abnormal differentiation and proliferation of lymphoid progenitors. Despite a significant improvement in relapse-free and overall survival for children with B-ALL, aB-ALL has a particularly poor prognosis with a 5-year survival rate of 20%. First remission is achieved for most patients, but relapse is common with a high associated mortality. New treatments such as immunotherapy offer an opportunity to extend remission and prevent relapse. Methods: aB-ALL antigens were identified using different sources—immunoscreening, protoarrays, two microarrays and one cancer-testis antigen database, and a review of the genomic analyses of aB-ALL. A total of 385 aB-ALL-associated gene products were examined for their association with patient survival. Results: We identified 87 transcripts with differential expression between aB-ALL and healthy volunteers (peripheral blood, bone marrow and purified $CD19^+$ cells), and 42 that were associated with survival. Enrichr analysis showed that the Transforming Growth Factor-β (TGFβ), Wnt and Hippo pathways were highly represented ($p < 0.02$). We found that SOX4 and ROCK1 were upregulated in all types of B-ALL (ROCK1 having a $p < 0.001$ except in t(8;14) patients), as well as SMAD3 and TEAD4 upregulation being associated with survival ($p = 0.0008$, 0.05 and 0.001, respectively). Expression of each aB-ALL antigen was verified by qPCR, but only TEAD4 showed significant transcript upregulation in aB-ALL compared to healthy volunteer CD19+ cells ($p = 0.01$). Conclusions: We have identified a number of

check for updates

Academic Editor: Constantin N. Baxevanis

Received: 16 February 2025
Revised: 20 March 2025
Accepted: 31 March 2025
Published: 22 April 2025

Citation: Mohamed, E.; Goodman, S.; Cooksey, L.; Fletcher, D.M.; Dean, O.; Boncheva, V.B.; Mills, K.I.; Orchard, K.H.; Guinn, B.-a. Adult B-Cell Acute Lymphoblastic Leukaemia Antigens and Enriched Pathways Identify New Targets for Therapy. *Onco* **2025**, *5*, 19. https://doi.org/10.3390/onco5020019

antigens and their pathways that play key roles in aB-ALL and may act as useful targets for future immunotherapy strategies.

Keywords: adult B-cell acute lymphocytic leukaemia; antigen discovery; cancer vaccines; SMAD3; TEAD4; immunotherapy

1. Introduction

Adult B-cell acute lymphoblastic leukaemia (aB-ALL) occurs due to an impairment in B-cell differentiation, leading to an accumulation of blast cells in the bone marrow. ALL has a bimodal distribution in terms of age, with peak incidences in children aged 2–5 years, and in adults (those aged 18 and over) when they are over 40 years of age. Adults over 60 account for 20% of ALL cases and 50% of ALL-related deaths [1]. Although early induction treatment can result in first remission for 80–90% of B-ALL patients, long-term survival is hampered by resistance, relapse, and extramedullary infiltration [2]. For this reason, the monitoring of minimal residual disease has been used to determine prognosis, best practice treatment options and risk of relapse for patients [3].

First-line treatment for aB-ALL involves pre-phase and induction therapy followed by consolidation and maintenance. Although 80–90% of aB-ALL patients achieve first remission, more than half relapse. In recent years, a number of immunotherapy strategies, including antibody and chimeric antigen receptor (CAR) T-cell therapies, have been used to break immunological tolerance and changed treatment outcomes for patients with relapsed/refractory (R/R) ALL following chemotherapy and haematopoietic stem cell transplant. Blinatumomab, a bispecific T cell engager that directs CTL to CD19-expressing cells, inotuzumab ozogamicin, an anti-CD22 antibody conjugated to calichaemicin and tisangeneucel, a CAR T cell therapy, have all achieved improved outcomes for patients with R/R ALL when compared to standard therapies [4–6]. Despite its efficacy, CAR-T cell therapy is associated with adverse effects, including B-cell aplasia, increased infection susceptibility due to the impairment of antibody production, and more serious events such as cytokine release syndrome leading to neurotoxicity and multiple organ dysfunction [7]. In addition, the time-consuming process of CAR-T cell engineering, antigenic variability due to ALL being a heterogeneous disease and trying to develop off-the-shelf therapies represent a real challenge [8].

Recently, the paradigm has shifted to immune-checkpoint inhibitors, although these can lead to excessive immune stimulation [9]. To address this issue, "armoured" CARs have been developed to secrete PD-1-blocking cytokines or co-express dominant-negative transforming growth factor-B receptor type II to create a proinflammatory environment [10]. However, long-term use of immunotherapy can lead to the selection of leukaemic clones that are resistant to treatment, leading to (for example, $CD19^{-}$) escape variants and relapse.

In addition to the immunotherapy targets supra vide, the identification of antigens associated with survival in leukaemia, especially for subtypes associated with poor prognosis and phenotypic plasticity, remains an attractive approach for B-ALL treatment. Seroprofiling of B-ALL, compared to age- and sex-matched healthy volunteer (HV) samples, revealed three differentially recognised tumour antigens, bone marrow tyrosine kinase (BMX), DCTPP1, and VGLL4 [11]. Targeting of BMX has been achieved using the epidermal growth factor receptor inhibitor, BMX-IN-1, and CTN06, which are small molecule inhibitors of both BMX and BTK, suppressing tumour growth and migration via the induction of autophagy and apoptosis. Ibrutinib and zanubrutinib are BTK inhibitors, the latter with improved specificity for BTK, and both of which have been used in clinical trials as

treatments for mature B-malignancies such as R/R chronic lymphoblastic leukaemia and small lymphocytic lymphoma [12]. Ibrutinib was found to cause a higher than expected level of adverse events and premature discontinuation in clinical trials while zanubrutinib has been found to have a more acceptable safety profile with improved response rates and progression-free survival [13,14].

Boullosa et al. [15] found that baculoviral IAP repeat containing 5 (*BIRC5*)/Survivin was expressed in aB-ALL but not HVs ($p = 0.015$). This anti-apoptotic protein is found at low levels in terminally differentiated healthy tissues but is upregulated in many cancers, such as lung and breast cancer, due to the high expression of oncogenes (JAK/STAT, Akt/PI3K and TCF-β-catenin pathways) and loss of tumour suppressor genes (P53 pathway) promoting tumour proliferation and survival [16]. BIRC5 overexpression is also associated with chemotherapy resistance and tumour aggression, while *BIRC5* knockdown results in the induction of apoptosis of leukaemia cells and increased chemosensitivity in vitro [17]. BIRC5 and BMX are newly identified tumour-associated antigens (TAAs) in B-ALL [11,15] and offer an opportunity for existing therapies to be repurposed to aB-ALL.

Due to the existence of immune escape variants, the heterogeneity between and within aB-ALL tumour cells, and the limitations with existing immunotherapy treatments (generating a lasting anti-tumour immune response), it is essential to broaden the number, specificity and sensitivity of TA targets available for future clinical use [18]. The search for leukaemia-associated and ideally cancer-restricted antigens that are effective targets to stimulate the immune destruction of aB-ALL cells may help in the development of new treatments that boost anti-leukaemia immune responses, while retaining the specificity needed to minimise off-target effects. To maximise the identification of new targets for treatment and enable the repurposing of already approved drugs for B-ALL patients, aB-ALL TAAs were identified from six sources—immunoscreening, protoarrays, two microarrays and one cancer-testis antigen (CTA) database, and a review of the genomic analyses of aB-ALL, and their relationship with patient survival was determined.

2. Materials and Methods

2.1. Patient Samples

This study received local ethical approval (REC Reference: 07/H0606/88; LREC 228/02/T), and samples were collected (Tables 1 and 2) following informed consent. On the day of sample receipt, serum was collected from clotted peripheral blood (PB) samples following centrifugation at 1200× *g* for 10 min. Bone marrow (BM) and PB samples were collected in K_2-EDTA tubes, incubated with five volumes of red cell lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA) for 30 min at room temperature. Leukocytes were isolated by centrifugation for 10 min at 800× *g*. HVs were self-selecting individuals who responded to a local request for samples. They had no signs of illness and were not undergoing any investigations by a health care professional. $CD19^+$ B-cells were used as a control, as pre-normal B-cells could not be sourced. $CD19^+$ is expressed on most B-ALL cells, normal pre-B, and B-cells up until maturation to plasma cells and was purchased from Cambridge Bioscience (Cambridge, UK) after isolation via negative immunomagnetic separation from leukophoresate. The donor was a female aged 18–66, and cells were shipped on dry ice. Isolated leukocytes were resuspended at 5×10^6/mL in PBS and 5 µL spotted onto two sites on each microscope slide. Once dried for 4–16 h, slides were saran wrapped and stored at −20 °C for future immunocytochemistry (ICC) analysis. Remaining leukocytes were pelleted and, along with sera samples, were immediately stored at −80 °C for later use. K562 (ATCC) and A549 (Sigma-Aldrich Co., Ltd., Gillingham, UK) were cultured in RPMI or DMEM, respectively, 10% foetal calf serum, 1% L-Glutamine and 1% penicillin streptomycin (all Sigma-Aldrich Co. Ltd.) in a 5% CO_2 in a humidified

chamber. K562 cells were used as a positive control for the expression of all antigens of interest in this study, except Yes-Associated Protein (YAP1), while A549 was used as a positive control for YAP1 expression.

Table 1. Patient information.

ID	Disease Stage	Cytogenetics	WCC (10^9/L)	BM Blast %	Relapse	Survival Post-Sample (mo) ¶	Age ≠	Sex	Sample Type
ALL001	Diagnosis Ph^+ ALL	Ph^+ ALL: t(9;22)	4.9	40	NK	NK	39	M	PB
ALL002	Diagnosis T-ALL	46,XY,t(1;7)(p36;p15)	232	88	No	Alive (post allo)	19	M	PB
ALL003	Diagnosis B-ALL	t(1;19)	28.6	91	No	Alive	26	F	PB
ALL004 *	Diagnosis T-ALL	Complex karyotype	591	NK	No	Died 19 mo (post allo)	19	M	PB
ALL005 *	¶ Post allo T-ALL	No result	NK	NK	No	Died 3.5 mo (post allo)	46	M	PB
ALL007	Diagnosis Pre-B-ALL	Loss of one copy of ETV6 (12p13) and gain of one copy of ABL1 (9q34) by FISH	8.2	92	No	Alive	24	M	PB
ALL008	Diagnosis Pre-B-ALL	46XY, 5,del(5)(q15q33),dic(9;16)(p11;q11), del(13)(q12q14)	10.4	72	No	Alive	19	M	PB
ALL009	Diagnosis Pre-B-ALL	46,XY,t(1;7)(q25;q3?5),add(3)(p1?3)	48.1	89.6	Yes	Died 94 mo (post allo, CART)	19	M	BM
ALL010 *	Diagnosis Pre-B-ALL	Complex including t(4;11)	229.1	85	Yes	Died 3 mo	64	M	PB
ALL011	Diagnosis Pre-B-ALL	No result—external referral	NK	NK	NK	NK	19	F	PB
ALL012	Diagnosis Pre-B-ALL	t(11;14)(q24;q32)	4.3	83	No	Alive (post allo)	33	M	BM
ALL014	Diagnosis Pre-B-ALL	47,XY,+2,add(2)(p1)[3]/46,XY [47]. nucish (CRLF2)x2[100]	4.9	53	Yes	Alive (post allo & CART)	56	M	BM
ALL015	Diagnosis Pre-B-ALL	Gain of one copy of CRLF2 (Xp22.3/Yp11.3) and loss of one copy of CSFR1 (5q32) and EBF1 (5q33.3) detected by FISH	68.0	94	No	Alive	20	F	PB
ALL016	Diagnosis Pre-B-ALL	Hyperdiploid; 56–57 XX +X,+4,+6,+9,+10,+14,+17,+18, +21,+marker	1.9	96	No	Alive	27	F	BM
ALL017 †	Diagnosis, Pre-B-ALL	No cytogenetics available, no FISH	3.0	96	No	Alive	19	F	PB
ALL018 †	Diagnosis, T-ALL	Normal cytogenetics, SET/CAN fusion detected by FISH	58.8	74	No	Alive	22	M	PB
ALL020	Diagnosis Pre-B-ALL	46,XY, t(1;7)(q25;q3?5), add(3)(p1?3) TCF3 ex16-PBX1 ex3 fusion transcript detected	31.9	92	Yes	Died 34.5 mo (Post allo, relapse, salvage chemo then CART & relapse)	56	F	PB/BM

≠—age at time of sampling; ¶—status on 30 October 2024; Allo: allogeneic stem cell transplant; NK: not known; *—samples used in immunoscreening, qPCR and ICC; †: serum samples used for immunoscreening only; ?—query to location of the translocation; []—indicate number of cells assessed as showing this rearrangement. The MILE study includes a limited number of samples from childhood B-ALL (cB-ALL) for C3 (c-ALL/pre-B-ALL with t(9;22)) and C8 (c-ALL/pre-B-ALL without t(9;22) which is known as Philadelphia (Ph) and Philadelphia-like ALL (Ph-like ALL) cytogenetics) subclasses [19] as well as a pre-ponderance of aB-ALL.

Table 2. HV demographics.

HV Control	Age ≠	Sex	Sample Type
HV008	40	F	PB
HV010	22	M	PB
HV012	46	F	PB
HV021	34	M	PB
HV043	NK	M	PB
$CD19^+$ cells	18–66	F	PB

≠: age at time of sampling; NK; not known; PB: peripheral blood.

2.2. Antigen Identification

Genes and antigens were identified from six sources as follows:-

(i) Serological analysis of recombinant cDNA expression libraries (SEREX)

The testis was chosen as the source tissue for the cDNA library due to its similarities in a broad range of gene expression by virtue of global promoter hypomethylation and increased chance of identifying CTAs. The testis cDNA library was made using the ZAP Express® cDNA synthesis and ZAP Express® cDNA Gigapack® III Gold cloning kit (Stratagene Europe) as described previously [20]. SEREX was performed following an optimised protocol [21] based on the original method [22]. The reactivity of serum was confirmed through agglutination tests, and $>10^6$ plaques in the testes cDNA library were immunoscreened using pre-cleared serum from five representative aALL patients (ALL004; ALL005; ALL010; ALL017; ALL018; Table 1). cDNA, in plaques that were reactive in two independent rounds of immunoscreening, were amplified using T3 and T7 primers and Ready Mix Taq PCR Reaction Mix with $MgCl_2$ (Sigma Aldrich, Ayrshire, UK). PCR products were gel-purified (QIAQuick gel extraction kit, Qiagen Ltd., Lancashire, UK), Sanger sequenced (Department of Biochemistry, University of Cambridge) and analysed using Applied Biosystems' Sequencing Analysis Software (StepOne software v2.0). Sequences were compared to expressed sequence tag (EST) and protein databases, including the National Centre for Biotechnology Information (NCBI) BLAST® > blastn suite, and these aALL antigens were added to the pool for survival and pathways analysis.

(ii) and (iii) Differentially expressed genes (DEGs) identified through the analysis of two microarray databases

DEGs in aB-ALL were identified using (i) the GSE38403 [23] and (ii) the Microarray Innovations in leukaemia (MILE; GSE13204) [24] microarray datasets via BloodSpot.eu. 649 aB-ALL samples and 74 HVs were utilised to compare gene transcription levels in ALL patient subgroups. Patient samples had been collected from 16 acute and chronic leukaemia subclasses, myelodysplastic syndromes (MDS), and a "none of the target classes" control group [19]. The read count was downloaded from GEO, and gene expression was calculated by the Fragments per Kilobase per Million Mapped Fragments (FPKM) method. Data were normalised using the limma method and DEGs were identified (fold change $\geq$1.5 for upregulated genes and $\leq$1.5 for downregulated ones) by the DESeq2 method, which is part of the 'Bioconductor' package. The adjusted p-value (qp) and p-value ≤ 0.05 were calculated using Fisher's test [25].

(iv) A review of genomic studies

We also analysed all of the genes identified by Iacobucci and Mullighan's [26] review of genomic studies. Genes with a corrected Spearman's correlation false detection rate (FDR) corrected to $p > 0.05$ were filtered out, and the remaining genes were arranged in accordance with decreasing Spearman's value, thus creating a ranked correlation file. These aB-ALL antigens were added to the pool of genes for survival and pathways analysis.

(v) Proto-array analysis

A total of 18 antigens had previously been identified using protoarrays, by virtue of their preferential recognition by antibodies in patients but not HV sera [11] and these were added to the pool of antigens for further analysis.

(vi) the CTA database

We were particularly interested in CTAs because of their cancer-restricted expression (recently reviewed in [27]). In total, 78 antigens identified in the CTA database [28,29] were added to the pool of aB-ALL antigens for survival and pathways analysis.

2.3. Pathway Analysis and Survival

The molecular pathways utilised by the antigens were determined using Enrichr [30,31] and the core genes in these selected pathways were examined for their protein–protein interaction networks using the Search Tool for the Retrieval of Interacting Genes (STRING) analytical program [32]. We determined which antigens, when transcribed at above and below median levels, were associated with aB-ALL patient survival using the MILE dataset accessed via bloodspot.eu [33]. This identified gene expression data from 649 B-ALL patients. We then priority-ranked the list of cancer antigens based on predefined and preweighted objective criteria following the analytical hierarchy process developed by a panel of 80 experts and described by Cheever et al. [34].

2.4. qPCR

RNA extraction was performed on 11 PB and 5 BM samples from 15 aALL patients and 6 PB samples from HVs (Table 2), including one sample of $CD19^+$ purified cells, using the RNeasy Mini Kit® (Qiagen) and following the manufacturer's guidelines. The genomic DNA elimination mix was prepared in a RNAse and DNAse-free PCR tube according to the manufacturer's instructions (MBI Fermentas). Quantitative PCR (qPCR) was performed using the RT^2 SYBR® Green Master-mix, RT^2 qPCR Primer Assay and cDNA synthesis reaction (all Qiagen) on a 96-well qPCR plate (Applied Biosystems, Foster city, CA, USA). TATA-box binding protein (*TBP1*) and protein kinase cGMP-dependent 1 (*PRKG1*) were used as reference genes following MIQE guidelines [35]. Primers from Qiagen were used to detect each transcript: SRY-Box Transcription Factor 4 (*SOX4*; PPH01950A), Rho associated coiled-coil containing protein kinase 1 (*ROCK1*; PPH01966C), *YAP1* (PPH13459A), TEA Domain Transcription Factor 4 (*TEAD4*; PPH10558A-200), SMAD family member 3 (*SMAD3*; PPH01921C), and T cell receptor 4 (*TCF4*; PPH02770A) and *BIRC5* (PH00271E). Every sample was analysed for expression of each gene in triplicate. To control for gDNA contamination within the qPCR reagents, a no-cDNA control was included on every qPCR plate, whereby cDNA was replaced by RNase-free H_2O. Otherwise, the reaction volumes were 10 µL 2× QuantiNova SYBR green PCR Mix, 2 µL QN ROX reference dye, 2 µL QuantiTect primer assay and 5 µL RNase-free H_2O, making a total volume of 19 µL added to each well in the 96-well plate. In a 1 µL final volume, ≤100 ng of template cDNA was added to achieve a final volume of 20 µL. The plate was then loaded into the thermocycler (StepOne Plus Real-Time PCR system, Applied Biosystems).

The cycle was 2 min at 95 °C, then 40 cycles of 5 s at 95 °C followed by 10 s at 60 °C. This was immediately followed by a melt curve stage of 15 s at 95 °C, 1 min at 60 °C, and 15 s at 95 °C, to verify the specificity of the amplification. Data were collected using StepOne software v2.0 (Applied Biosystems) and the results were normalised using the TBP1 and PRKG1 housekeeping genes (ΔCT = CT genes of interest−CT housekeeping genes). The $2^{-\Delta\Delta CT}$ method [36] log transforms the ΔΔCT (where ΔΔCT = ΔCT patient sample−ΔCT HV) to determine the relative expression of the seven genes of interest (BIRC5, ROCK1, SMAD3, SOX4, TCF4, TEAD4, and YAP1).

2.5. Immunocytochemistry

Immunocytochemistry was performed as described previously [37] using monoclonal rabbit anti-human antibodies against TEAD4 (1D10) and SMAD3 (2C12) (ThermoFisher Scientific, Leicestershire, UK) (Table S1). Mouse anti-human monoclonal Actin antibody (ACTN05 (C4), Fisher Scientific) was used as a positive control for immunolabelling, with BIRC5 as a comparator [15], and isotype controls (mouse or rabbit anti-human IgG antibodies) were used to test for non-specific antibody binding. Secondary antibodies

were provided as part of the EnVision+ System/HRP, Rabbit(DAB+) kit (DAKO, via Bio Analytical, Chatterlis, UK). Staining intensities were scored according to a five-tiered scale described previously [38] as follows:—0: no staining; 1: background; 2: weak staining; 3: moderate staining; 4: strong staining. The percentage of positively stained cells was based on the cell count of stained cells per microscopic view represented on a five-tiered scale (0: 0%; 1: 1–10%; 2: 11–50%; 3: 51–80%; 4: >80%). The final immunoreactivity score was obtained by multiplication of the percentage of positively stained cells scored over ≥5 microscopic views by the value for staining intensity within the same [39].

3. Results

3.1. Immunoscreening Identified 72 aB-ALL Associated Antigens

Primary immunoscreening of the testes cDNA library with pre-cleared aB-ALL patient sera identified 310 sero-positive plaques. In total, 134 phage colonies were confirmed as expressing immunogenic polypeptides through a second independent round of immunoscreening. PCR amplification of cDNA inserts identified 69 independent known genes (Table S2) and three novel sequences (UOH-ALL-104, UOH-ALL-105, UOH-ALL-106). The function and distribution of the identified genes in cancer and healthy tissues were determined (Table S3) through a comprehensive search of the literature. The expression of SEREX-identified genes was examined using data from the MILE study and compared to bone marrow from HVs from the same (Table S3).

3.2. Association Between Leukaemia-Associated Antigen (LAA) Expression in aB-ALL Cells and Patient Survival

Of the 385 genes identified from the six sources ((i) SEREX; (ii) GSE38403; (iii) GSE13204; (iv) Iacobucci and Mullighan's review of genomic studies [26]; (v) proto-array analysis; and (vi) CTA database; Figure 1), 87 transcripts were found to have differential expression (Figure 2) and 42 of these had expression levels (above and below media) that were associated with survival in aB-ALL patients, determined using the MILE study data (Table S4).

3.3. TGFβ, Wnt and Hippo Pathways Were Highly Represented by the DEGs in aB-ALL Samples

Transforming Growth Factor-β (TGFβ), Wnt and Hippo pathways were highly represented by the genes ($p \leq 0.001$, ≤0.01 and ≤0.01, respectively), when analysed by Enrichr (Figure 3). The core genes from these pathways (TGFβ, Wnt, and Hippo pathways) were then examined using the MILE dataset for their association with patient survival ($n = 11$) (Table S4) and literature searches were performed to identify genes known to be involved in B-ALL. SOX4 and YAP1 were added to the antigen ranking as SOX4 was considered to be non-canonical activator for three pathways [40] whereas YAP1 has restricted expression in healthy tissues (excluding stem/progenitor populations) [41].

STRING showed that PMP2, PMP4, CDKN2B, RBX, CUL1 were involved in TGFβ enrichment (Figure S1) while Wnt2B, CDKN2B, RBX, PMP2, PTGS2 were involved in cancer pathways. SEREX identified genes that were involved in ubiquitination including UBC, UBE2C, UBE2D2, and CUL1 and genes involved in regulating the intrinsic apoptosis process (BCLAF2, PARL, SERINC3, TRAP1, TPT1, MCL1, and PTPN1).

Enrichr showed that the SEREX-identified antigens were mostly involved in molecular functions involving binding and catalytic activity, and the Wiki pathway (Table 3). In addition, envelope proteins (TCF7L2, Wnt2B), essential to maintaining the structure and function of the nucleus, mainly correlated with Emery–Dreifuss muscular dystrophy (EDMD). From MSigDB Hallmark 2020, the mitotic spindle pathway had a high p-value of 0.001, and this pathway plays a crucial role in cell division and chromosomal separation.

Using PANTHER.db, the most enriched pathway was the Cadherin signalling pathway (14.3%) and PTPN1, TCF7L2, FER, and Wnt2B were also identified by SEREX.

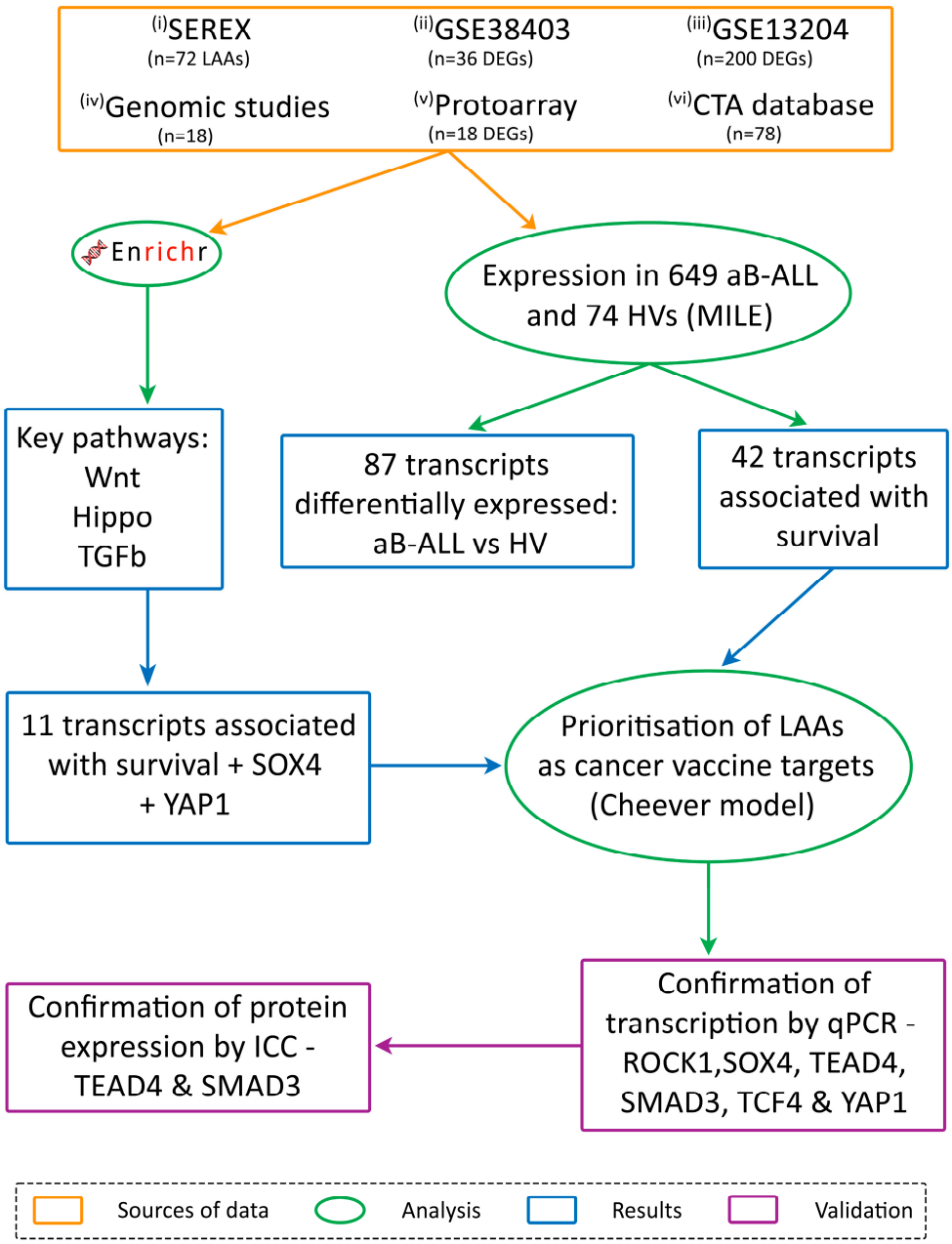

Figure 1. Workflow for the identification and analysis of aB-ALL antigens from six sources. Identification of antigens methods included (i) SEREX; (ii + iii) Differentially expressed genes; (iv) review of genomic studies (v) proto-array analysis and (vi) the CTA database. Antigens were analysed for their differential expression, expression in gene pathways and association with survival. Antigens were prioritised using the model devised by Cheever et al. [34]. From the final antigens analysed for RNA transcription and translation, we found SMAD3 and TEAD4 to be elevated in aB-ALL samples compared to HV samples. Gold box indicates sources of data, green oval indicates analysis steps, blue boxes indicate where results were generated, and violet boxes where validation steps were performed.

3.4. Most of the aB-ALL Antigens Were Differentially Expressed in Solid Tumours

Of the antigens that were ranked by the Cheever system [34], ubiquitin C (HMG20/UBC) and MOB kinase activator 1B (MOB1B) were rarely detected in the healthy

tissues (Figure S2A), while SOX4 was found at low to moderate levels in most solid tumours. ROCK1 and YAP1 had very similar patterns of expression in solid tumours, except lymphomas, where ROCK1 was upregulated and YAP1 was not detected. SMAD3 was upregulated in renal cancers. TGFβ1 was not found in most solid tumours or found at low levels, while TCF4, HMG20, and septin 9 (SEPT 9) were not found in any of the solid tumours investigated (Figure S2B).

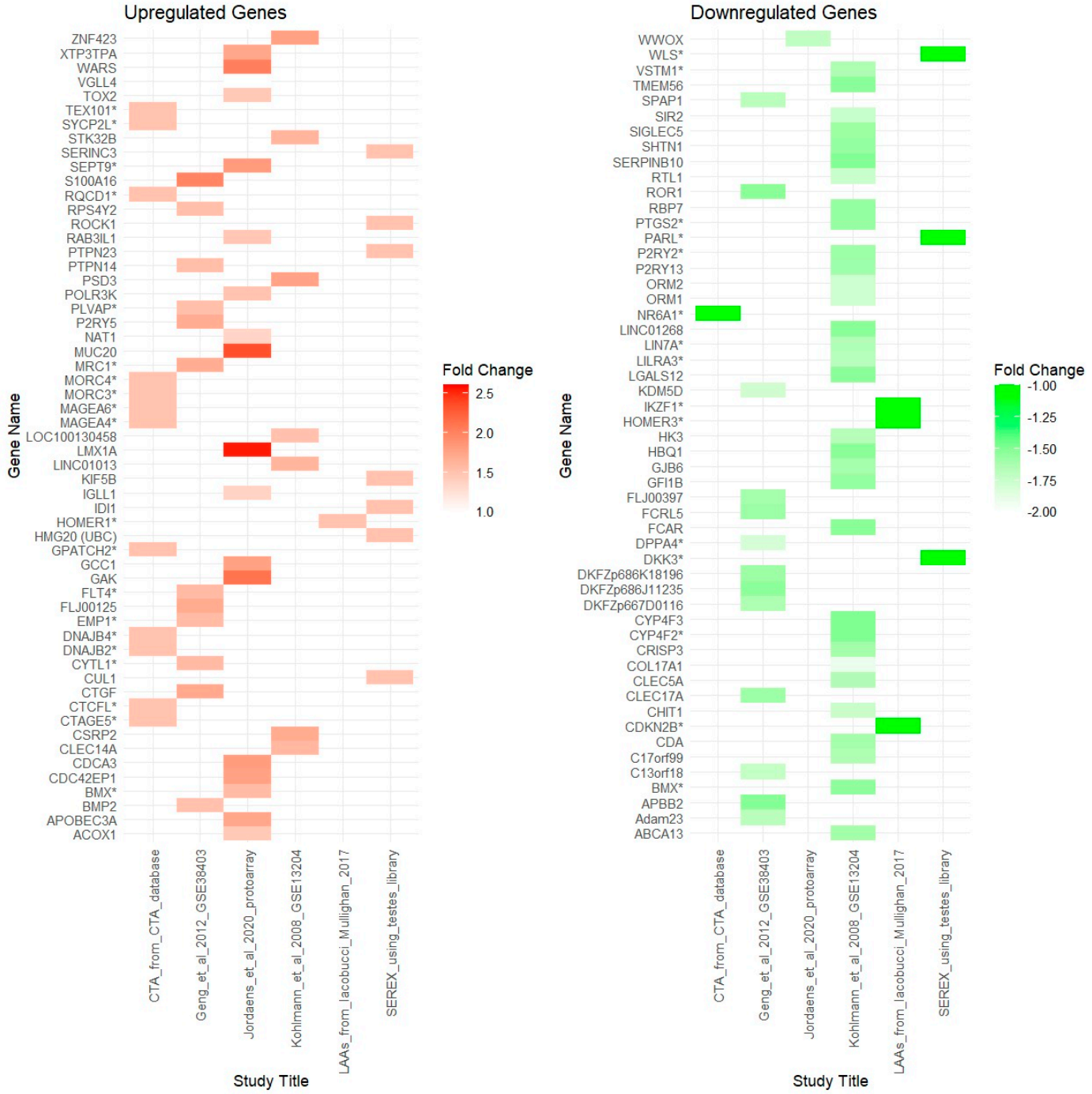

Figure 2. Relative expression of aB-ALL associated genes from each source. Differentially expressed genes which were upregulated (red) or downregulated (green) are shown as a fold change (FC) and by a key, ranging in FC from +1 to +2.5 and −1 to −2, respectively. The * indicates genes that were associated with survival ($p < 0.05$). Only BMX was common between two studies (protoarray and MILE study).

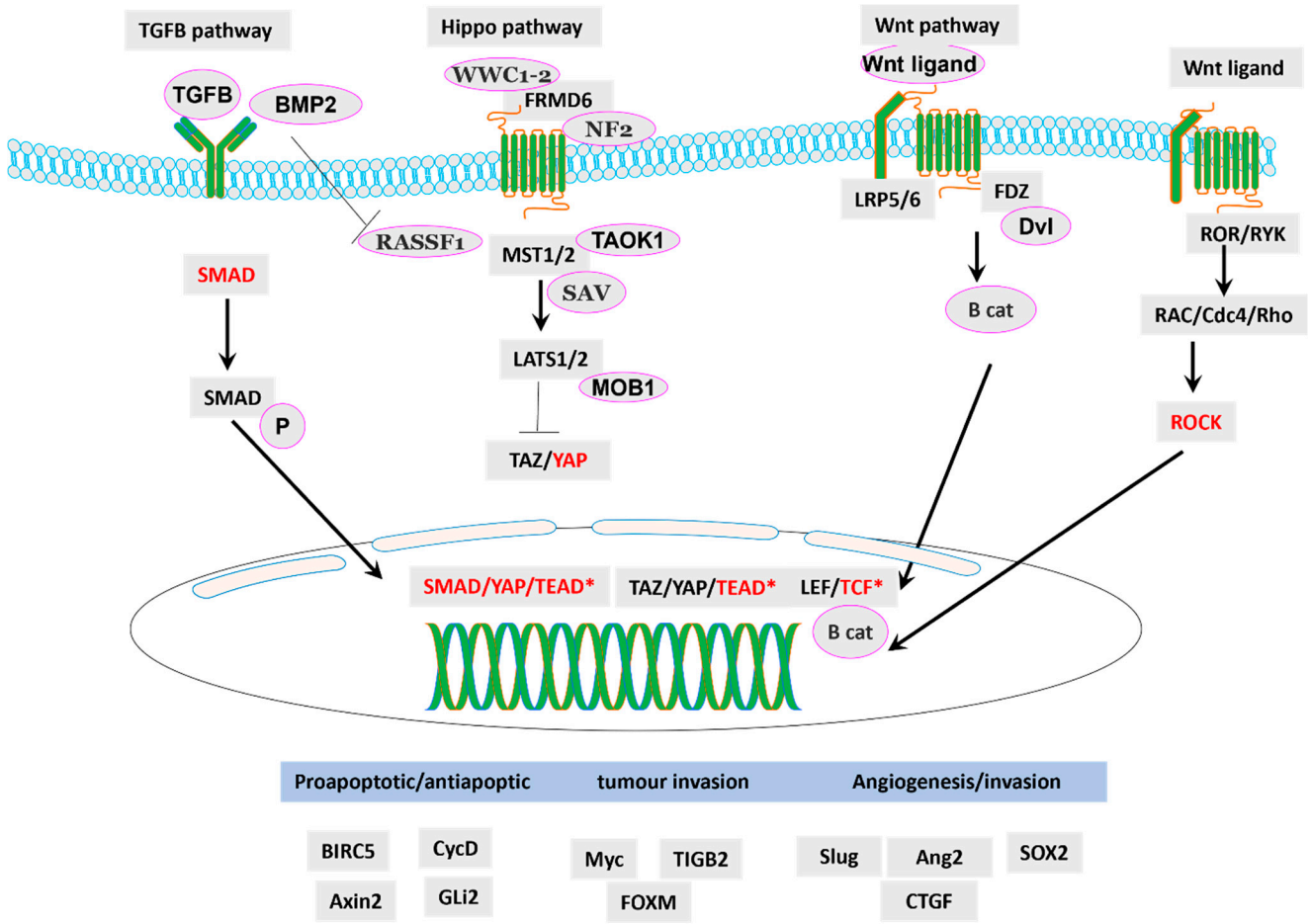

Figure 3. Role of gene products in key pathways and prioritising of gene products to act as vaccine targets. Three key pathways were found to be enriched within the list of genes identified (genes identified shown in red text). These were the Hippo, TGFβ, and Wnt pathways each involved in promoting leukaemogenesis via the upregulation of anti-apoptotic genes and pro-proliferative genes that causes blasts to continue growing and resist to apoptosis. Genes that were ranked highly by the Cheever et al. 2009 [34] criteria (as indicated by an asterix) were selected for verification of their expression in B-ALL samples versus normal donors.

Table 3. Pathways identified as involving members of the SEREX-identified genes.

Pathway	*p*-Value	q-Value	SEREX-Identified Genes Involved in This Pathway	Database Used
Envelope proteins and their potential roles in EDMD physiopathology	0.0007	0.0892	TCF7L2, WNT2B	Wiki Pathway
Hematopoietic stem cell gene regulation	0.001	0.064	DNMT1, MCL1	
TGFβ signalling pathway	0.015	0.229	TRAP1, ROCK1, CUL1	
Mitotic spindle	0.001	0.038	ROCK1, KIF5B, FLNB, KIF1B, MYH10	MSigDB Hallmark 2020
Notch signalling	0.007	0.117	TCF7L2, CUL1	
Apoptosis	0.026	0.286	ROCK1, TIMP1, MCL1	

3.5. SOX4 and TGFβ1 Were the Top-Ranking aB-ALL Vaccine Targets

aB-ALL antigens that impacted survival were ranked using the criteria described by Cheever et al. [34] to prioritise cancer antigens on their capacity to act as vaccine targets in clinical studies (Figure 4). SOX4 (0.89) and TGFβ1 (0.81) had the top scores. while ROCK1, YAP1, TEAD4, SMAD3, and TCF4 had the next highest cumulative scores of 0.41, 0.36,

0.34, 0.33, and 0.32, respectively. TGFβ1 was downregulated in aB-ALL compared to HV samples ($p < 0.0001$), reflecting its activity as a tumour suppressor [42] and was excluded from further analysis.

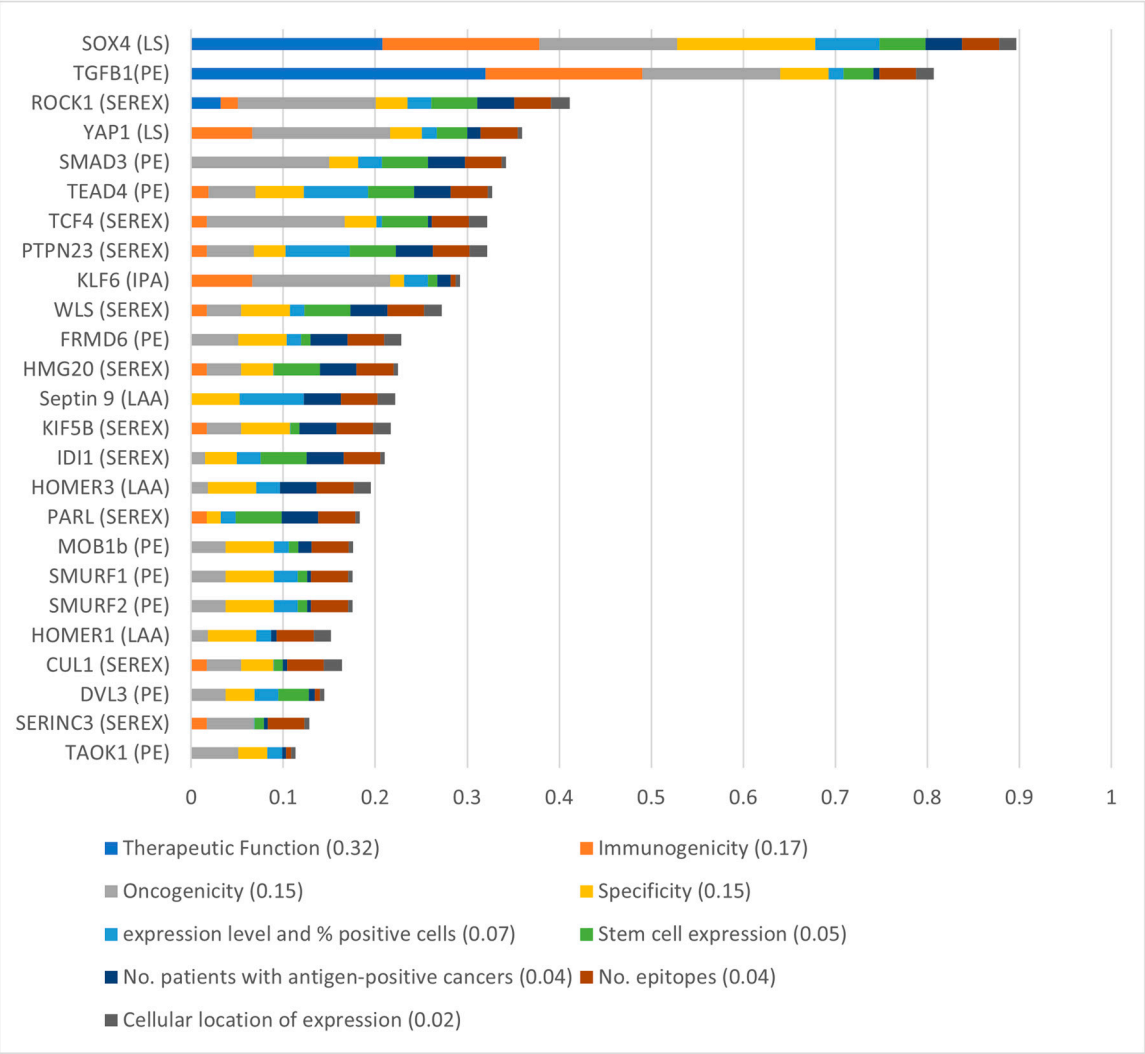

Figure 4. Antigens that were identified using SEREX, pathway enrichment (PE), literature search (LS), CTA database, and microarrays (LAA). Key shows evaluation criteria and the maximum attainable score in parentheses; Genes indicated in red font were ranked highly within the Cheever system [34] and were selected for further analysis.

3.6. TCF4, SOX4 and SMAD3 Transcription Was Increased in Almost All aB-ALL Subtypes

We analysed the expression levels of the six most highly ranked aB-ALL antigens (SOX4, ROCK1, TCF4, TEAD4, SMAD3, and YAP1), identified using the Cheever system [34], and used BIRC5 as a comparator, in 16 aALL PB and BM samples compared to four HV PB and one $CD19^+$ sample by qPCR. The K562 cell line was used as a positive control for *BIRC5, TCF4, SMAD3*, and *ROCK1* expression, while A549 cells were used as a positive control for *YAP1*. Transcription of all aALL-antigens was elevated, while BIRC5, SMAD3, and TEAD4 had the highest mean fold change in aALL patient samples compared to HV samples (Figure 5A).

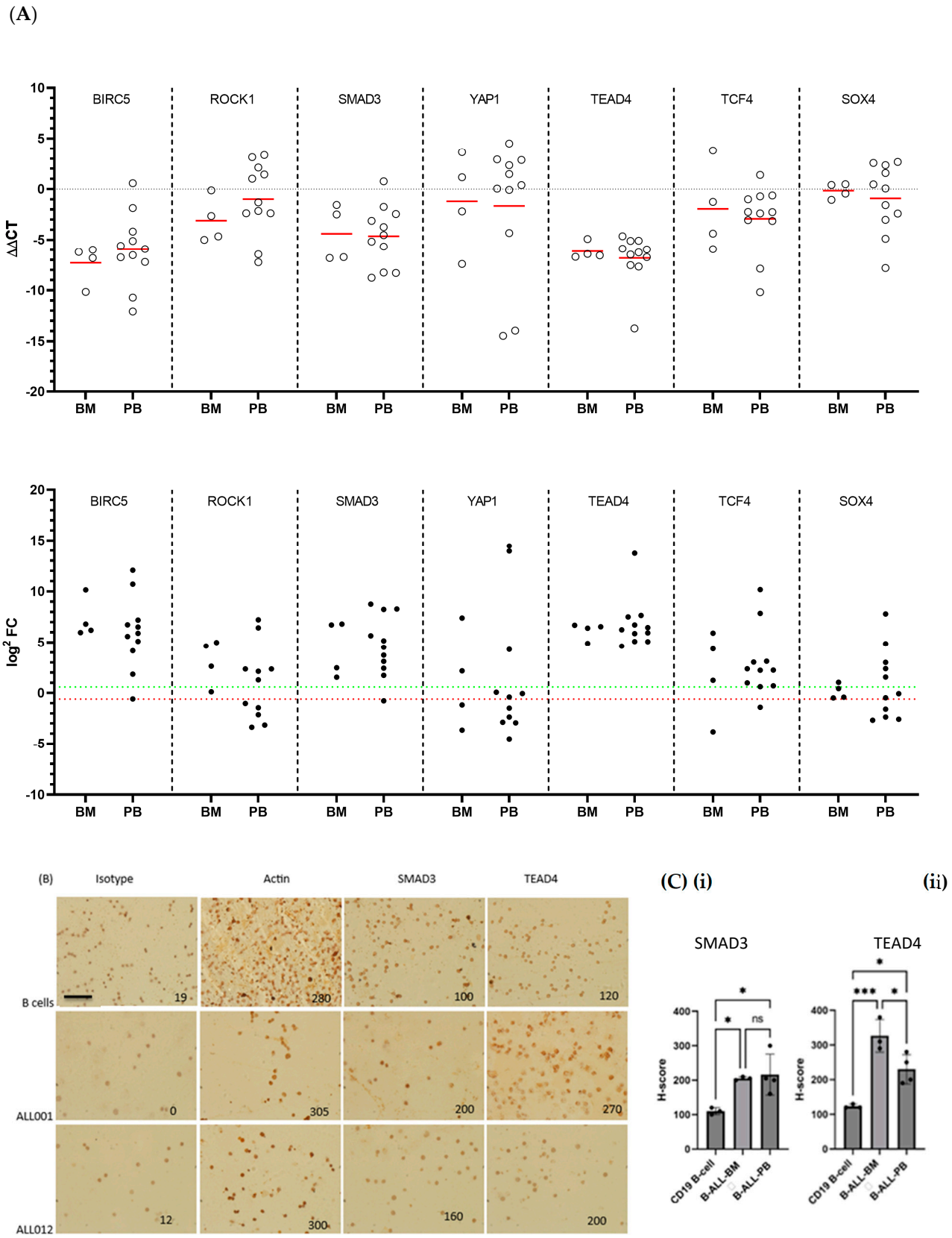

Figure 5. Expression of the key genes in aALL patient samples. (**A**) ΔΔCT and log-transformed $2^{-\Delta\Delta CT}$ was used to determine relative gene expression of *BIRC5*; *ROCK1*; *SMAD3*; *SOX4*; *TCF4*; *TEAD4*, and *YAP1* when compared to the average of two reference genes—*TBP* and *PRKG1*. Red bars represent mean ΔΔCT, and relative expression thresholds were set at 0.6 $\log^2$FC (green dotted line) and −0.6 $\log^2$Expression was analysed in 11 PB and 5 BM samples from ALL patients and five PB samples

from HVs using a one-way ANOVA. (**B**) Immunolabelling of SMAD3 and TEAD4 in one HV $CD19^+$ B-cells and six patient samples. The immunoreactivity score (0–400) is shown in black text on the bottom right corner of each image, and the size bar is 20 µm. Images were taken at 400× magnification. Isotype caused little immunolabelling while actin provided a positive control for immunolabelling (stained dark brown). (**C**) (**i**) SMAD3 and (**ii**) TEAD4 immunolabelling were detected in all patient samples with immunoreactivity scores (*y*-axis) of 200–350, indicating their varied but elevated levels in aB-ALL samples when compared to $CD19^+$ cells from the PB of an HV using a Dunnett test. NS: not significant; *: significant at $p < 0.05$ and *** significant at $p < 0.001$.

In the MILE study, *ROCK1* was found to be upregulated in all types of aB-ALL ($p < 0.05$), except t(8;14)/t(1;19)/Pre-B-ALL t(9;22), which were not significant (NS). *TEAD4* was upregulated in aB-ALL with t(8;14), t(1;19), c-/Pre-B-ALL t(9;22)—$p < 0.001$. There was no consistent difference in *YAP1* expression between aB-ALL and HV samples, with variation occurring depending on the probe set used. TCF4 was upregulated in all subtypes of aB-ALL— $p < 0.001$, *SOX4* was upregulated in all subtypes of B-ALL—$p < 0.001$ except t(8;14) and *SMAD3* was upregulated in all subtypes of aB-ALL- $p < 0.05$.

3.7. TEAD4 and SMAD3 Protein Expression Was Elevated in aB-ALL Samples

To examine SMAD3 and TEAD4 expression, ICC was performed on six B-ALL samples compared to one sample of HV $CD19^+$ B-cells. TEAD4 and SMAD3 levels were significantly higher in aB-ALL samples compared to HV $CD19^+$ B-cells ($p < 0.05$; Figure 5B). TEAD4 protein was mostly found in the nucleus, whereas SMAD3 was found in both the nucleus and cytoplasm. TEAD4 protein levels were significantly different between PB and BM. We found no statistical significance in immunoreactivity scores between TEAD4, SMAD3, and historical BIRC5 data when we compared the levels of TEAD4 and SMAD3 in this study and BIRC5 levels in the same three patient samples analysed in our previous study [15].

4. Discussion

A number of immunotherapy strategies have been used to improve treatment outcomes for patients with R/R ALL following chemotherapy and haematopoietic stem cell transplant. However, long-term use of immunotherapy can lead to the selection of leukaemic clones that are resistant to treatment. This evolutionary process hampers the effectiveness of immunotherapy by selecting subclones that are unresponsive to current therapy, resulting in escape variants and relapse. To overcome their existence, the heterogeneity between and within aB-ALL tumour cells, and the limitations with existing immunotherapy treatments, it is essential to broaden the number, specificity and sensitivity of tumour antigen targets available for future clinical use. To this end, we immunoscreened a testes cDNA library with sera from five representative aALL sera. We identified 134 seropositive plaques that encoded 72 independent sequences and three cDNAs (UOH-ALL-104, UOH-ALL-105, and UOH-ALL-106) did not map to known genes, providing a source of previously uncharacterised gene products for future studies.

The majority of SEREX-identified antigens (67/72) had already been shown to be involved in tumour pathogenesis, four were CTAs (TUBA3C/UOH-ALL-3, C10orf82/UOH-ALL-20, CT152/UOH-ALL-83, and PRM1/UOH-ALL-101), three were highly enriched in the testis and involved in spermatogenesis (TEX43/UOH-ALL-43, CCDC89/UOH-ALL-49, and LOC338963/UOH-ALL-85) and three were non-coding RNAs that had testis-restricted expression (LINC00661/UOH-ALL-58, LOC338963/UOH-ALL-85, and LINC00251/UOH-ALL-98).

In addition, we collated CTAs and LAAs from our previous studies [11] and that of others [23,24,26,28,29], determined the pathways most often utilised by the antigens and the core genes in these selected pathways, and then examined the association between

levels of expression as determined by the MILE study in aB-ALL samples with survival. Levels of *SOX4* and *YAP1* expression were not associated with survival, while *TGFβ1*, *ROCK1* and *TEAD4* were. We found that *SOX4* and *TEAD4* were upregulated in aB-ALL patient samples, while lower *SOX4* expression showed a trend towards better outcomes and survival ($p = 0.07$). SOX4 belongs to the sex-determining region Y-related HMG box family. Its abnormal expression is associated with cancer, reflecting its role in regulating cell stemness, proliferation and differentiation in healthy cells. SOX4 is known to regulate several cancer-promoting signalling pathways, including Wnt, TGFβ and PI3K [43], and the TGFβ. Wnt and Hippo pathways were all highly represented by the aB-ALL antigens. SEREX also identified ROCK1/UOH-ALL-65, which has been shown previously to regulate normal haematopoiesis through the negative regulation of erythropoietic stress and inflammation [44]. ROCK1 is an established oncogene in AML, where its overexpression is associated with poor survival ($p < 0.01$) [45]. Of note, using data from the MILE study, we identified *TEAD4*, *SMAD3*, *SOX4*, and *TCF4* as being upregulated in cB-ALL compared to HVs, while *ROCK1* and *YAP1* were not. *ROCK1* oncogenes may be an attractive target for future treatments, as a small molecule inhibitor, GSK269962A, has already been investigated in an AML mouse model [46]. GSK269962A was shown to inhibit tumour growth, apoptosis and clonogenicity, significantly prolonging survival with some mice remaining disease-free for more than 140 days. Knocking out *ROCK1* was shown to at least double the survival time in a KIT(D814V)-induced myeloid leukaemia model when compared to controls [47]. The anti-proliferative activity of GSK269962A occurs via the inhibition of cell cycle kinase (CDK6), which induces cell cycle arrest. It also causes apoptosis via increased expression and phosphorylation of p53 as well as decreased expression of antiapoptotic genes, including survivin, Bcl-xL, and it has been shown to cause the cleavage of PARP in AML cells [46].

TEAD4, the second element of the Hippo pathway, is activated by co-regulators such as YAP, TAZ, p160, and VGLL-4. The epithelial-mesenchymal transition is driven by YAP, and the transcription factor TEAD is essential for cell division. Normal cell growth requires that TEAD-YAP activity be tightly regulated. In quiescent adult normal cells, the Hippo route closely regulates YAP through cell–cell interaction. But during neoplastic transformation, YAP is frequently dysregulated, which results in increased and carcinogenic TEAD-YAP activity. Through the overexpression of target genes like CTGF and CYR61, this aberrant activity promotes cell proliferation and transformation, which aids in the aetiology of a number of human malignancies [41].

TGFβ signalling in haemopoietic and lymphoid cells is complex and context-dependent [48]. The TGFβ1 pathway is involved in a variety of cellular functions, including inhibiting cell proliferation, and is often inactivated in lymphoma and acute myeloid leukaemia [49]. Although the MILE study showed that *TGFβ1* was downregulated in aB-ALL, in contrast to paediatric B-ALL [50], TGFβ1 acts as a potent immune suppressant that inhibits NK effector function, and TGFβ blocking monoclonal antibodies have been shown to reverse ALL-mediated NK cell dysfunction [50]. In contrast, adding exogenous TGFβ1 to HV NK cells induced an inhibitory phenotype similar to ALL-NK cells, which has been shown to act through the SMAD signalling pathway [50].

The Wnt/β-catenin pathway regulates cell proliferation, survival and differentiation in hematopoietic stem cells (HSCs), while deregulation of this pathway leads to haematological malignancies, including B-ALL [51]. SEREX identified four Wnt pathway components (WLS, Wnt2b, TCF7L2, and DKK3) that were recognised by antibodies in aB-ALL patient sera. Targeting Wnt ligands and receptors can reduce leukaemic stemness [52] while knockdown of *FZD6/WNT10B* reduced the proliferation of leukaemic stem cells [53]. However, TGFβ and NFAT pathways sustain Wnt activation, increasing the leukaemic burden [53].

KIBRA is a promising tumour suppressor in drosophila and mammalian cells [54] and an upstream regulator of the Hippo pathway. It was found to be frequently silenced due to hypermethylation in cB-ALL [55], suggesting one mode by which the Hippo pathway could be targeted.

We also identified eukaryotic initiation factor 2α (eIF2α) through the immunoscreening of a testis cDNA library using ALL sera. The activation of protein kinase RNA-like endoplasmic reticulum kinase (PERK) leads to the phosphorylation of eIF2α. This phosphorylation event promotes the translation of activating transcription factor 4 (ATF4) while reducing overall protein synthesis [56]. ATF4, as a transcription factor, has the ability to increase the expression of CCAAT/enhancer-binding protein homologous protein (CHOP). ATF4 and CHOP together can also upregulate the production of growth arrest and DNA damage-inducible protein-34 (GADD34), which acts to dephosphorylate phospho-eIF2α [56]. Therefore, prolonged endoplasmic reticulum (ER) stress, without the restoration of protein synthesis homeostasis, can ultimately result in apoptotic cell death, which may be worthy of further investigation in the context of treating aB-ALL.

Our investigation of genes that can act as possible targets for immunotherapy has identified antigens that are involved in patient survival (ROCK1, TEAD4, SMAD3, and TCF4), in maintaining tumour stem cell features (SOX4, YAP1, and TCF4), as well as the tumour microenvironment (ROCK1, YAP1, and TEAD4). Several of the antigens we identified have been found to play a role in childhood B-ALL (SMAD3 [57]; TCF4 [58]) and demonstrate the broad potential of treatments targeting these proteins/pathways.

The identification of immunogenic epitopes within antigens and their capacity to stimulate the immune response is the usual next step in the development of immunotherapy strategies for patient treatment [59,60]. However, there is a need to break immune tolerance, which is exacerbated by the immunosuppressive microenvironment created by tumour cells through the secretion of IL-10 and TGFβ [61]. This can be enhanced through the treatment of patients with immune checkpoint inhibitors such as anti-PD-1, which have been shown in vitro to enhance anti-leukaemic stem cell activity [62]. The direct targeting of proteins such as TEAD4 and SMAD3 may be especially challenging because they are involved in the maintenance of homeostatic functions and may cause significant off-target effects. To mitigate this, antigens can be targeted by specific and efficacious delivery, for example, through the use of bi-specific antibodies, bispecific T cell engagers and CAR-T cells.

Additional investigations using small inhibitors/protein–protein interaction targeting are necessary to determine the clinical utility of direct targeting and the specificity of such treatment for diseased cells [63]. Although BIRC5 is a novel target, the phase I trial using antisense oligonucleotide targeting BIRC5 has been examined in children who had relapsed with B-ALL (cB-ALL). However, the trial was discontinued due to high toxicity and off-target effects [17]. One reason for this limited success may be that many immunodominant TAAs are self-proteins. As such, they are subject to both central and peripheral tolerances that dampen immune responses to avoid autoimmunity. This situation demonstrates the need to break self-tolerance in a controlled manner, replicating stem cell transplant strategies that have been used and evolved to effectively treat at least a proportion of patients with haematological malignancies since the 1930s.

5. Limitations of the Study

SEREX is often viewed as an outdated method since the advent of quicker methods to identify antigen expression in specific cell types. However, the immunoscreening of a testes cDNA library allows the identification of novel (as yet undefined) antigens, and although laborious, complements our group's existing experience in using SEREX [20,21,64,65].

We priority-ranked the antigens using the system described by Cheever et al. [34] that assesses antigens for their utility as vaccine targets, however, this mathematical model has not been updated since 2009, has the disadvantage of giving a high weighting to therapeutic function and may not be precise in reflecting the fairness and reliability of the data [34,66]. However, in the absence of better criteria, we used the model to identify the five aB-ALL antigens that scored highest for vaccine target potential and the correlation of their expression with patient survival.

The scarcity of samples from aB-ALL patients for qPCR and ICC studies reflects the scarcity of patients who are affected by aB-ALL and the period of time in which RNA can be stored at −80 °C and cells on microscope slides at −20 °C. To account for this, we analysed the expression of the genes in aB-ALL samples using the BloodSpot.eu database that collated data from 649 aB-ALL patients.

6. Conclusions

Current treatments for aB-ALL have involved integrating chemotherapy and targeted therapy. aB-ALL is a complex disease with various factors influencing the overall prognosis, including cytogenetic abnormalities, MDR, and response to therapy. Simply targeting the tumour antigens on and in malignant cells is not enough to eradicate tumour growth, as the tumour microenvironment within the BM plays a crucial role in treatment outcomes. Sensitivity to immunotherapy in aB-ALL is not solely determined by intrinsic biological factors but also by the diverse interactions between leukaemia cells and the bone marrow microenvironment [63]. Leukaemia cells exploit this microenvironment to sustain their proliferation and survival, taking advantage of the tightly controlled signalling pathways and transcriptional factors that regulate normal lymphopoiesis. Breaking immune tolerance towards leukaemic cells will require a multifaceted approach. The identification of further targets for immunotherapy, as performed in this study, provides an opportunity to develop personalised therapies and additional targets that can be used to minimise the opportunities for escape mutants to survive the treatment process and cause relapse.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/onco5020019/s1, Table S1. Antibodies used for ICC; Table S2. Characterisation of cDNA sequences confirmed by two rounds of immunoscreening; Table S3. Expression of SEREX-identified genes and their function in health and cancer tissues [67–203]; Table S4. Gene expression and association with above and below median expression using MILE data; Figure S1. Protein-protein interaction of LAAs using STRING. Most of the LAAs were involved in binding followed by transcription regulator activity. PMP2, PMP4, CDKN2B, RBX, CUL1 involved in TGFβ enrichment. Wnt2B, CDKN2B, RBX, PMP2, PTGS2 were involved in cancer pathways. The lack of interactions among other genes in this signature is likely explained by a variety of other biological pathways utilised within B-ALL. SEREX identified genes that were involved in ubiquitination included UBC, UBE2C, UBE2D2, CUL1, RBX1, and SKP2. Genes involved in regulating the intrinsic apoptosis process were BCLAF2, PARL, SERINC3, TRAP1, TPT1, MCL1, and PTPN1; Figure S2. In silico analysis of the antigens revealed the levels of expression of each shortlisted antigen in healthy and cancerous tissues. Heatmap showing the expression levels of each protein in (A) healthy cells and (B) different tumour types, with the y-axes representing the proportion of patients expressing each tumour antigen. The purple, red, and orange colours indicate very high to low levels of expression, pale yellow represents no expression detected, and white represents no data available in the corresponding histology in the Human Protein Atlas.

Author Contributions: Conceptualisation, E.M. and B.-a.G.; methodology, E.M. and B.-a.G.; validation, E.M., S.G. and L.C.; formal analysis, E.M., S.G. and L.C.; investigation, E.M., S.G. and L.C.; resources, V.B.B. and K.H.O.; data curation, E.M., D.M.F., K.I.M. and K.H.O.; writing—original draft preparation, E.M., S.G., L.C. and B.-a.G.; writing—review and editing, E.M., O.D. and B.-a.G.; visual-

isation, E.M. and B.-a.G.; supervision, B.-a.G.; project administration, B.-a.G.; funding acquisition, B.-a.G. All authors have read and agreed to the published version of the manuscript.

Funding: E.M. and L.C. were funded by University of Hull cluster PhD studentships and V.B. by Breakthrough Cancer Research (previously known as Cork Cancer Research Centre).

Institutional Review Board Statement: Informed consent and the collection of aB-ALL samples were performed according to the guidelines of the Declaration of Helsinki, and approved by a Local Ethics Committee in the UK as well as the Southampton University Hospitals Trust and University of Hull ethics committees.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We would like to thank the patients for their kind donation of samples.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

aB-ALL	Adult B-cell acute lymphoblastic leukaemia
BIRC5	baculoviral IAP repeat containing 5
BMX	bone marrow tyrosine kinase
CAR	Chimeric antigen receptor
cB-ALL	children with B-ALL
CTA	cancer-testis antigen
DEGs	Differentially expressed genes
HV	Healthy volunteer
LAA	leukaemia-associated antigens
NS	Not significant
PB	Peripheral blood
PRKG1	protein kinase cGMP-dependent 1
R/R	relapsed/refractory
ROCK1	Rho-associated coiled-coil containing protein kinase 1
SMAD3	SMAD family member 3
SOX4	SRY-Box Transcription Factor 4
TAAs	Tumour-associated antigens
TBP1	TATA-box binding protein
TCF4	T cell Factor 4
TEAD4	TEA Domain Transcription Factor 4
TGFβ	Transforming Growth Factor-β
YAP1	Yes-associated protein1

References

1. Samra, B.; Jabbour, E.; Ravandi, F.; Kantarjian, H.; Short, N.J. Evolving therapy of adult acute lymphoblastic leukemia: State-of-the-art treatment and future directions. *J. Hematol. Oncol.* **2020**, *13*, 70. [CrossRef]
2. DeAngelo, D.J.; Jabbour, E.; Advani, A. Recent advances in managing acute lymphoblastic leukemia. *Am. Soc. Clin. Oncol. Educ. Book.* **2020**, *40*, 330–342. [CrossRef]
3. Gokbuget, N.; Dombret, H.; Giebel, S.; Bruggemann, M.; Doubek, M.; Foa, R.; Hoelzer, D.; Kim, C.; Martinelli, G.; Parovichnikova, E.; et al. Minimal residual disease level predicts outcome in adults with Ph-negative B-precursor acute lymphoblastic leukemia. *Hematology* **2019**, *24*, 337–348. [CrossRef] [PubMed]

4. Kantarjian, H.M.; Vandendries, E.; Advani, A.S. Inotuzumab Ozogamicin for Acute Lymphoblastic Leukemia. *N. Engl. J. Med.* **2016**, *375*, 2100–2101. [CrossRef] [PubMed]
5. Brown, P.A.; Ji, L.; Xu, X.; Devidas, M.; Hogan, L.E.; Borowitz, M.J.; Raetz, E.A.; Zugmaier, G.; Sharon, E.; Bernhardt, M.B.; et al. Effect of Postreinduction Therapy Consolidation With Blinatumomab vs. Chemotherapy on Disease-Free Survival in Children, Adolescents, and Young Adults With First Relapse of B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. *JAMA* **2021**, *325*, 833–842. [CrossRef]
6. Stackelberg, A.V.; Jaschke, K.; Jousseaume, E.; Templin, C.; Jeratsch, U.; Kosmides, D.; Steffen, I.; Gokbuget, N.; Peters, C. Tisagenlecleucel vs. historical standard of care in children and young adult patients with relapsed/refractory B-cell precursor acute lymphoblastic leukemia. *Leukemia* **2023**, *37*, 2346–2355. [CrossRef] [PubMed]
7. Paul, S.; Kantarjian, H.; Jabbour, E.J. Adult acute lymphoblastic leukemia. In *Mayo Clinic Proceedings*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 1645–1666.
8. Martino, M.; Alati, C.; Canale, F.A.; Musuraca, G.; Martinelli, G.; Cerchione, C. A Review of Clinical Outcomes of CAR T-Cell Therapies for B-Acute Lymphoblastic Leukemia. *Int. J. Mol. Sci.* **2021**, *22*, 2150. [CrossRef]
9. Webster, J.; Luskin, M.R.; Prince, G.T.; DeZern, A.E.; DeAngelo, D.J.; Levis, M.J.; Blackford, A.; Sharon, E.; Streicher, H.; Luznik, L. Blinatumomab in combination with immune checkpoint inhibitors of PD-1 and CTLA-4 in adult patients with relapsed/refractory (R/R) CD19 positive B-cell acute lymphoblastic leukemia (ALL): Preliminary results of a phase I study. *Blood* **2018**, *132*, 557. [CrossRef]
10. Namuduri, M.; Brentjens, R.J. Enhancing CAR T cell efficacy: The next step toward a clinical revolution? *Expert. Rev. Hematol.* **2020**, *13*, 533–543. [CrossRef]
11. Jordaens, S.; Cooksey, L.; Freire Boullosa, L.; Van Tendeloo, V.; Smits, E.; Mills, K.I.; Orchard, K.H.; Guinn, B.A. New targets for therapy: Antigen identification in adults with B-cell acute lymphoblastic leukaemia. *Cancer Immunol. Immunother.* **2020**, *69*, 867–877. [CrossRef]
12. Coutre, S.E.; Byrd, J.C.; Hillmen, P.; Barrientos, J.C.; Barr, P.M.; Devereux, S.; Robak, T.; Kipps, T.J.; Schuh, A.; Moreno, C.; et al. Long-term safety of single-agent ibrutinib in patients with chronic lymphocytic leukemia in 3 pivotal studies. *Blood Adv.* **2019**, *3*, 1799–1807. [CrossRef] [PubMed]
13. Brown, J.R.; Eichhorst, B.; Hillmen, P.; Jurczak, W.; Kazmierczak, M.; Lamanna, N.; O'Brien, S.M.; Tam, C.S.; Qiu, L.; Zhou, K.; et al. Zanubrutinib or Ibrutinib in Relapsed or Refractory Chronic Lymphocytic Leukemia. *N. Engl. J. Med.* **2023**, *388*, 319–332. [CrossRef]
14. Hillmen, P.; Eichhorst, B.; Brown, J.R.; Lamanna, N.; O'Brien, S.M.; Tam, C.S.; Qiu, L.; Kazmierczak, M.; Zhou, K.; Simkovic, M.; et al. Zanubrutinib Versus Ibrutinib in Relapsed/Refractory Chronic Lymphocytic Leukemia and Small Lymphocytic Lymphoma: Interim Analysis of a Randomized Phase III Trial. *J. Clin. Oncol.* **2023**, *41*, 1035–1045. [CrossRef]
15. Boullosa, L.F.; Savaliya, P.; Bonney, S.; Orchard, L.; Wickenden, H.; Lee, C.; Smits, E.; Banham, A.H.; Mills, K.I.; Orchard, K. Identification of survivin as a promising target for the immunotherapy of adult B-cell acute lymphoblastic leukemia. *Oncotarget* **2018**, *9*, 3853. [CrossRef]
16. Li, Y.; He, W.; Gao, X.; Lu, X.; Xie, F.; Um, S.-W.; Kang, M.-W.; Yang, H.; Shang, Y.; Wang, Z. Cullin7 induces docetaxel resistance by regulating the protein level of the antiapoptotic protein Survivin in lung adenocarcinoma cells. *J. Thorac. Dis.* **2023**, *15*, 5006–5019. [CrossRef] [PubMed]
17. Li, F.; Aljahdali, I.; Ling, X. Cancer therapeutics using survivin BIRC5 as a target: What can we do after over two decades of study? *J. Exp. Clin. Cancer Res.* **2019**, *38*, 368. [CrossRef]
18. Valipour, B.; Abedelahi, A.; Naderali, E.; Velaei, K.; Movassaghpour, A.; Talebi, M.; Montazersaheb, S.; Karimipour, M.; Darabi, M.; Chavoshi, H.; et al. Cord blood stem cell derived CD16+ NK cells eradicated acute lymphoblastic leukemia cells using with anti-CD47 antibody. *Life Sci.* **2020**, *242*, 117223. [CrossRef]
19. Haferlach, T.; Kohlmann, A.; Wieczorek, L.; Basso, G.; Kronnie, G.T.; Béné, M.-C.; De Vos, J.; Hernández, J.M.; Hofmann, W.-K.; Mills, K.I. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: Report from the International Microarray Innovations in Leukemia Study Group. *J. Clin. Oncol.* **2010**, *28*, 2529–2537. [CrossRef] [PubMed]
20. Boncheva, V.B.; Linnebacher, M.; Kdimati, S.; Draper, H.; Orchard, L.; Mills, K.I.; O'Sullivan, G.; Tangney, M.; Guinn, B.-a. Identification of the Antigens Recognised by Colorectal Cancer Patients Using Sera from Patients Who Exhibit a Crohn's-like Lymphoid Reaction. *Biomolecules* **2022**, *12*, 1058. [CrossRef]
21. Liggins, A.P.; Guinn, B.A.; Banham, A.H. Identification of lymphoma-associated antigens using SEREX. *Methods Mol. Med.* **2005**, *115*, 109–128.
22. Sahin, U.; Tureci, O.; Schmitt, H.; Cochlovius, B.; Johannes, T.; Schmits, R.; Stenner, F.; Luo, G.; Schobert, I.; Pfreundschuh, M. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 11810–11813. [CrossRef] [PubMed]

23. Geng, H.; Brennan, S.; Milne, T.A.; Chen, W.-Y.; Li, Y.; Hurtz, C.; Kweon, S.-M.; Zickl, L.; Shojaee, S.; Neuberg, D. Integrative epigenomic analysis identifies biomarkers and therapeutic targets in adult B-acute lymphoblastic leukemia. *Cancer Discov.* **2012**, *2*, 1004–1023. [CrossRef]
24. Kohlmann, A.; Kipps, T.J.; Rassenti, L.Z.; Downing, J.R.; Shurtleff, S.A.; Mills, K.I.; Gilkes, A.F.; Hofmann, W.K.; Basso, G.; Dell'orto, M.C.; et al. An international standardization programme towards the application of gene expression profiling in routine leukaemia diagnostics: The Microarray Innovations in LEukemia study prephase. *Br. J. Haematol.* **2008**, *142*, 802–807. [CrossRef] [PubMed]
25. Akhmedov, M.; Martinelli, A.; Geiger, R.; Kwee, I. Omics Playground: A comprehensive self-service platform for visualization, analytics and exploration of Big Omics Data. *NAR Genom. Bioinform.* **2020**, *2*, lqz019. [CrossRef] [PubMed]
26. Iacobucci, I.; Mullighan, C.G. Genetic basis of acute lymphoblastic leukemia. *J. Clin. Oncol.* **2017**, *35*, 975. [CrossRef]
27. Naik, A.; Lattab, B.; Qasem, H.; Decock, J. Cancer testis antigens: Emerging therapeutic targets leveraging genomic instability in cancer. *Mol. Ther. Oncol.* **2024**, *32*, 200768. [CrossRef]
28. Almeida, L.G.; Sakabe, N.J.; deOliveira, A.R.; Silva, M.C.; Mundstein, A.S.; Cohen, T.; Chen, Y.T.; Chua, R.; Gurung, S.; Gnjatic, S.; et al. CTdatabase: A knowledge-base of high-throughput and curated data on cancer-testis antigens. *Nucleic Acids Res.* **2009**, *37*, 816–819. [CrossRef]
29. Mohamed, E. *Identification of Tumour Antigens That May Facilitate Effective Cancer Detection and Treatment*; University of Hull: Hull, UK, 2023.
30. Chen, E.Y.; Tan, C.M.; Kou, Y.; Duan, Q.; Wang, Z.; Meirelles, G.V.; Clark, N.R.; Ma'ayan, A. Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinform.* **2013**, *14*, 128. [CrossRef]
31. Kuleshov, M.V.; Jones, M.R.; Rouillard, A.D.; Fernandez, N.F.; Duan, Q.; Wang, Z.; Koplev, S.; Jenkins, S.L.; Jagodnik, K.M.; Lachmann, A.; et al. Enrichr: A comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* **2016**, *44*, W90–W97. [CrossRef]
32. Szklarczyk, D.; Gable, A.L.; Lyon, D.; Junge, A.; Wyder, S.; Huerta-Cepas, J.; Simonovic, M.; Doncheva, N.T.; Morris, J.H.; Bork, P. STRING v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **2019**, *47*, D607–D613. [CrossRef]
33. Gislason, M.H.; Demircan, G.S.; Prachar, M.; Furtwangler, B.; Schwaller, J.; Schoof, E.M.; Porse, B.T.; Rapin, N.; Bagger, F.O. BloodSpot 3.0: A database of gene and protein expression data in normal and malignant haematopoiesis. *Nucleic Acids Res.* **2024**, *52*, D1138–D1142. [CrossRef]
34. Cheever, M.A.; Allison, J.P.; Ferris, A.S.; Finn, O.J.; Hastings, B.M.; Hecht, T.T.; Mellman, I.; Prindiville, S.A.; Viner, J.L.; Weiner, L.M.; et al. The prioritization of cancer antigens: A national cancer institute pilot project for the acceleration of translational research. *Clin. Cancer Res.* **2009**, *15*, 5323–5337. [CrossRef] [PubMed]
35. Lossos, I.S.; Czerwinski, D.K.; Wechser, M.A.; Levy, R. Optimization of quantitative real-time RT-PCR parameters for the study of lymphoid malignancies. *Leukemia* **2003**, *17*, 789–795. [CrossRef] [PubMed]
36. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
37. Khan, G.; Brooks, S.; Mills, K.; Guinn, B. Infrequent expression of the cancer–testis antigen, PASD1, in ovarian cancer. *Biomark. Cancer* **2015**, *7*, 31–38. [CrossRef] [PubMed]
38. Biesterfeld, S.; Veuskens, U.; Schmitz, F.; Amo-Takyi, B.; Böcking, A. Interobserver reproducibility of immunocytochemical estrogen-and progesterone receptor status assessment in breast cancer. *Anticancer. Res.* **1996**, *16*, 2497–2500.
39. Deng, Z.; Hasegawa, M.; Aoki, K.; Matayoshi, S.; Kiyuna, A.; Yamashita, Y.; Uehara, T.; Agena, S.; Maeda, H.; Xie, M. A comprehensive evaluation of human papillomavirus positive status and p16INK4a overexpression as a prognostic biomarker in head and neck squamous cell carcinoma. *Int. J. Oncol.* **2014**, *45*, 67–76. [CrossRef]
40. Mehta, G.A.; Angus, S.P.; Khella, C.A.; Tong, K.; Khanna, P.; Dixon, S.A.; Verzi, M.P.; Johnson, G.L.; Gatza, M.L. SOX4 and SMARCA4 cooperatively regulate PI3k signaling through transcriptional activation of TGFBR2. *NPJ Breast Cancer* **2021**, *7*, 40. [CrossRef]
41. Steinhardt, A.A.; Gayyed, M.F.; Klein, A.P.; Dong, J.; Maitra, A.; Pan, D.; Montgomery, E.A.; Anders, R.A. Expression of Yes-associated protein in common solid tumors. *Hum. Pathol.* **2008**, *39*, 1582–1589. [CrossRef]
42. Mostufi-Zadeh-Haghighi, G.; Veratti, P.; Zodel, K.; Greve, G.; Waterhouse, M.; Zeiser, R.; Cleary, M.L.; Lübbert, M.; Duque-Afonso, J. Functional Characterization of Transforming Growth Factor-β Signaling in Dasatinib Resistance and Pre-BCR+ Acute Lymphoblastic Leukemia. *Cancers* **2023**, *15*, 4328. [CrossRef]
43. Luo, X.; Ji, X.; Xie, M.; Zhang, T.; Wang, Y.; Sun, M.; Huang, W.; Xia, L. Advance of SOX transcription factors in hepatocellular carcinoma: From role, tumor immune relevance to targeted therapy. *Cancers* **2022**, *14*, 1165. [CrossRef] [PubMed]
44. Mali, R.S.; Kapur, S.; Kapur, R. Role of Rho kinases in abnormal and normal hematopoiesis. *Curr. Opin. Hematol.* **2014**, *21*, 271. [CrossRef] [PubMed]

45. Liu, Y.; Gao, X.; Tian, X. High expression of long intergenic non-coding RNA LINC00662 contributes to malignant growth of acute myeloid leukemia cells by upregulating ROCK1 via sponging microRNA-340-5p. *Eur. J. Pharmacol.* **2019**, *859*, 172535. [CrossRef]
46. Pan, T.; Wang, S.; Feng, H.; Xu, J.; Zhang, M.; Yao, Y.; Xu, K.; Niu, M. Preclinical evaluation of the ROCK1 inhibitor, GSK269962A, in acute myeloid leukemia. *Front. Pharmacol.* **2022**, *13*, 1064470. [CrossRef]
47. Mali, R.S.; Ramdas, B.; Ma, P.; Shi, J.; Munugalavadla, V.; Sims, E.; Wei, L.; Vemula, S.; Nabinger, S.C.; Goodwin, C.B.; et al. Rho kinase regulates the survival and transformation of cells bearing oncogenic forms of KIT, FLT3, and BCR-ABL. *Cancer Cell* **2011**, *20*, 357–369. [CrossRef]
48. Tamayo, E.; Alvarez, P.; Merino, R. TGFβ superfamily members as regulators of B cell development and function—Implications for autoimmunity. *Int. J. Mol. Sci.* **2018**, *19*, 3928. [CrossRef] [PubMed]
49. Vicioso, Y.; Gram, H.; Beck, R.; Asthana, A.; Zhang, K.; Wong, D.P.; Letterio, J.; Parameswaran, R. Combination Therapy for Treating Advanced Drug-Resistant Acute Lymphoblastic Leukemia. *Cancer Immunol. Res.* **2019**, *7*, 1106–1119. [CrossRef]
50. Rouce, R.H.; Shaim, H.; Sekine, T.; Weber, G.; Ballard, B.; Ku, S.; Barese, C.; Murali, V.; Wu, M.F.; Liu, H.; et al. The TGF-β/SMAD pathway is an important mechanism for NK cell immune evasion in childhood B-acute lymphoblastic leukemia. *Leukemia* **2016**, *30*, 800–811. [CrossRef]
51. Khoury, H.; Pinto, R.; Meng, Y.; He, R.; Wu, S.; Minden, M.D. Noggin Overexpression Enhances Leukemic Progenitors Self-Renewal in AML by Abrogating the BMP Pathway Activation. *Blood* **2006**, *108*, 2214. [CrossRef]
52. Chiarini, F.; Paganelli, F.; Martelli, A.M.; Evangelisti, C. The Role Played by Wnt/β-Catenin Signaling Pathway in Acute Lymphoblastic Leukemia. *Int. J. Mol. Sci.* **2020**, *21*, 1098. [CrossRef]
53. Cassaro, A.; Grillo, G.; Notaro, M.; Gliozzo, J.; Esposito, I.; Reda, G.; Trojani, A.; Valentini, G.; Di Camillo, B.; Cairoli, R.; et al. FZD6 triggers Wnt–signalling driven by WNT10BIVS1 expression and highlights new targets in T-cell acute lymphoblastic leukemia. *Hematol. Oncol.* **2021**, *39*, 364–379. [CrossRef]
54. Yu, J.; Zheng, Y.; Dong, J.; Klusza, S.; Deng, W.-M.; Pan, D. Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. *Dev. Cell* **2010**, *18*, 288–299. [CrossRef]
55. Hill, V.K.; Dunwell, T.L.; Catchpoole, D.; Krex, D.; Brini, A.T.; Griffiths, M.; Craddock, C.; Maher, E.R.; Latif, F. Frequent epigenetic inactivation of KIBRA, an upstream member of the Salvador/Warts/Hippo (SWH) tumor suppressor network, is associated with specific genetic event in B-cell acute lymphocytic leukemia. *Epigenetics* **2011**, *6*, 326–332. [CrossRef]
56. Fun, X.H.; Thibault, G. Lipid bilayer stress and proteotoxic stress-induced unfolded protein response deploy divergent transcriptional and non-transcriptional programmes. *Biochim. Biophys. Acta BBA-Mol. Cell Biol. Lipids* **2020**, *1865*, 158449. [CrossRef]
57. Liu, S.X.; Xiao, H.R.; Wang, G.B.; Chen, X.W.; Li, C.G.; Mai, H.R.; Yuan, X.L.; Liu, G.S.; Wen, F.Q. Preliminary investigation on the abnormal mechanism of $CD4^{+}FOXP3^{+}CD25^{high}$ regulatory T cells in pediatric B-cell acute lymphoblastic leukemia. *Exp. Ther. Med.* **2018**, *16*, 1433–1441.
58. Takahashi, H.; Kajiwara, R.; Kato, M.; Hasegawa, D.; Tomizawa, D.; Noguchi, Y.; Koike, K.; Toyama, D.; Yabe, H.; Kajiwara, M.; et al. Treatment outcome of children with acute lymphoblastic leukemia: The Tokyo Children's Cancer Study Group (TCCSG) Study L04-16. *Int. J. Hematol.* **2018**, *108*, 98–108. [CrossRef]
59. Backert, L.; Kohlbacher, O. Immunoinformatics and epitope prediction in the age of genomic medicine. *Genome Med.* **2015**, *7*, 119. [CrossRef]
60. Caron, E.; Aebersold, R.; Banaei-Esfahani, A.; Chong, C.; Bassani-Sternberg, M. A case for a human immuno-peptidome project consortium. *Immunity* **2017**, *47*, 203–208. [CrossRef]
61. Thomas, D.A.; Massagué, J. TGF-β directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* **2005**, *8*, 369–380. [CrossRef]
62. Greiner, J.; Goetz, M.; Schuler, P.J.; Bulach, C.; Hofmann, S.; Schrezenmeier, H.; Döhner, H.; Schneider, V.; Guinn, B.A. Enhanced stimulation of antigen-specific immune responses against nucleophosmin 1 mutated acute myeloid leukaemia by an anti-programmed death 1 antibody. *Br. J. Haematol.* **2022**, *198*, 866–874. [CrossRef]
63. Brown, G. Lessons to cancer from studies of leukemia and hematopoiesis. *Front. Cell Dev. Biol.* **2022**, *10*, 993915. [CrossRef]
64. Guinn, B.A.; Bland, E.A.; Lodi, U.; Liggins, A.P.; Tobal, K.; Petters, S.; Wells, J.W.; Banham, A.H.; Mufti, G.J. Humoral detection of leukaemia-associated antigens in presentation acute myeloid leukaemia. *Biochem. Biophys. Res. Commun.* **2005**, *335*, 1293–1304. [CrossRef]
65. Liggins, A.P.; Guinn, B.A.; Hatton, C.S.; Pulford, K.; Banham, A.H. Serologic detection of diffuse large B-cell lymphoma-associated antigens. *Int. J. Cancer* **2004**, *110*, 563–569, Erratum in *Int. J. Cancer* **2004**, *110*, 934. [CrossRef]
66. Silva, W.A.; Gnjatic, S.; Ritter, E.; Chua, R.; Cohen, T.; Hsu, M.; Jungbluth, A.A.; Altorki, N.K.; Chen, Y.-T.; Old, L.J. PLAC1, a trophoblast-specific cell surface protein, is expressed in a range of human tumors and elicits spontaneous antibody responses. *Cancer Immun. Arch.* **2007**, *7*, 18.
67. Watkins, P.A.; Maiguel, D.; Jia, Z.; Pevsner, J. Evidence for 26 distinct acyl-coenzyme A synthetase genes in the human genomes. *J. Lipid Res.* **2007**, *48*, 2736–2750. [CrossRef]

68. Zhao, Z.; Liu, M.; Xu, Z.; Cai, Y.; Peng, B.; Liang, Q.; Yan, Y.; Liu, W.; Kang, F.; He, Q.; et al. Identification of ACSF gene family as therapeutic targets and immune-associated biomarkers in hepatocellular carcinoma. *Aging* **2022**, *14*, 7926–7940. [CrossRef]
69. Wang, Y.; Wo, Y.; Lu, T.; Sun, X.; Liu, A.; Dong, Y.; Du, W.; Su, W.; Huang, Z.; Jiao, W. Circ-AASDH functions as the progression of early stage lung adenocarcinoma by targeting miR-140-3p to activate E2F7 expression. *Transl. Lung Cancer Res.* **2021**, *10*, 57–70. [CrossRef]
70. Tan, Y.; You, H.; Wu, C.; Altomare, D.A.; Testa, J.R. Appl1 is dispensable for mouse development, and loss of Appl1 has growth factor-selective effects on Akt signaling in murine embryonic fibroblasts. *J. Biol. Chem.* **2010**, *285*, 6377–6389. [CrossRef]
71. Thomas, R.M.; Nechamen, C.A.; Mazurkiewicz, J.E.; Ulloa-Aguirre, A.; Dias, J.A. The adapter protein APPL1 links FSH receptor to inositol 1,4,5-trisphosphate production and is implicated in intracellular Ca^{2+} mobilization. *Endocrinology* **2011**, *152*, 1691–1701. [CrossRef]
72. Zhai, J.S.; Song, J.G.; Zhu, C.H.; Wu, K.; Yao, Y.; Li, N. Expression of APPL1 is correlated with clinicopathologic characteristics and poor prognosis in patients with gastric cancer. *Curr. Oncol.* **2016**, *23*, e95–e101. [CrossRef]
73. Hupalowska, A.; Pyrzynska, B.; Miaczynska, M. APPL1 regulates basal NF-κB activity by stabilizing NIK. *J. Cell science* **2012**, *125*, 4090–4102. [CrossRef]
74. Wang, Y.; Tong, X.; Li, G.; Li, J.; Deng, M.; Ye, X. Ankrd17 positively regulates RIG-I-like receptor (RLR)-mediated immune signaling. *Eur. J. Immunol.* **2012**, *42*, 1304–1315. [CrossRef]
75. Dong, L.; Lin, F.; Wu, W.; Huang, W.; Cai, Z. Transcriptional cofactor Mask2 is required for YAP-induced cell growth and migration in bladder cancer cell. *J. Cancer* **2016**, *7*, 2132–2138. [CrossRef]
76. Wilkinson, S. ER-phagy: Shaping up and destressing the endoplasmic reticulum. *FEBS J.* **2019**, *286*, 2645–2663. [CrossRef]
77. Behrendt, L.; Hoischen, C.; Kaether, C. Disease-causing mutated ATLASTIN 3 is excluded from distal axons and reduces axonal autophagy. *Neurobiol. Dis.* **2021**, *155*, 105400. [CrossRef] [PubMed]
78. Waragai, M.; Nagamitsu, S.; Xu, W.; Li, Y.J.; Lin, X.; Ashizawa, T. Ataxin 10 induces neuritogenesis via interaction with G-protein β2 subunit. *J. Neurosci. Res.* **2006**, *83*, 1170–1178. [CrossRef]
79. Schäfer, M.; Oeing, C.U.; Rohm, M.; Baysal-Temel, E.; Lehmann, L.H.; Bauer, R.; Volz, H.C.; Boutros, M.; Sohn, D.; Sticht, C.; et al. Ataxin-10 is part of a cachexokine cocktail triggering cardiac metabolic dysfunction in cancer cachexia. *Mol. Metab.* **2016**, *5*, 67–78. [CrossRef]
80. Garcia-Gonzalo, F.R.; Corbit, K.C.; Sirerol-Piquer, M.S.; Ramaswami, G.; Otto, E.A.; Noriega, T.R.; Seol, A.D.; Robinson, J.F.; Bennett, C.L.; Josifova, D.J. A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nat. Genet.* **2011**, *43*, 776–784. [CrossRef]
81. Hopp, K.; Heyer, C.M.; Hommerding, C.J.; Henke, S.A.; Sundsbak, J.L.; Patel, S.; Patel, P.; Consugar, M.B.; Czarnecki, P.G.; Gliem, T.J.; et al. B9D1 is revealed as a novel Meckel syndrome (MKS) gene by targeted exon-enriched next-generation sequencing and deletion analysis. *Hum. Mol. Genet.* **2011**, *20*, 2524–2534. [CrossRef] [PubMed]
82. Song, C.-Q.; Li, Y.; Mou, H.; Moore, J.; Park, A.; Pomyen, Y.; Hough, S.; Kennedy, Z.; Fischer, A.; Yin, H. Genome-wide CRISPR screen identifies regulators of mitogen-activated protein kinase as suppressors of liver tumors in mice. *Gastroenterology* **2017**, *152*, 1161–1173.e1161. [CrossRef]
83. Lee, Y.; Yu, Y.; Gunawardena, H.; Xie, L.; Chen, X. BCLAF1 is a radiation-induced H2AX-interacting partner involved in γH2AX-mediated regulation of apoptosis and DNA repair. *Cell Death Disease* **2012**, *3*, e359. [CrossRef] [PubMed]
84. White, L.S.; Soodgupta, D.; Johnston, R.L.; Magee, J.A.; Bednarski, J.J. Bclaf1 Promotes Maintenance and Self-Renewal of Fetal Hematopoietic Stem Cells. *Blood* **2018**, *132*, 1269. [CrossRef]
85. Almutairi, M.; Alrubie, T.; Alamri, A.; Almutairi, B.; Alrefaei, A.; Arafah, M.; Alanazi, M.; Semlali, A. Cancer-Testis Gene Biomarkers discovered in Colon Cancer Patients. *Genes* **2022**, *13*, 807. [CrossRef]
86. Matsuda, A.; Suzuki, Y.; Honda, G.; Muramatsu, S.; Matsuzaki, O.; Nagano, Y.; Shimotohno, K.; Harada, T.; Nishida, E.; Hayashi, H. Large-scale identification and characterization of human genes that activate NF-κB and MAPK signaling pathways. *Oncogene* **2003**, *22*, 3307–3318. [CrossRef]
87. Salian, S.; Guo, X.Y.; Murakami, Y.; Kinoshita, T.; Kaur, P.; Shukla, A.; Girisha, K.M.; Fujita, M.; Campeau, P.M. C18orf32 loss-of-function is associated with a neurodevelopmental disorder with hypotonia and contractures. *Hum. Genet.* **2022**, *141*, 1423–1429. [CrossRef] [PubMed]
88. Malik, M.; Jividen, K.; Padmakumar, V.; Cataisson, C.; Li, L.; Lee, J.; Howard, O.Z.; Yuspa, S.H. Inducible NOS-induced chloride intracellular channel 4 (CLIC4) nuclear translocation regulates macrophage deactivation. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 6130–6135. [CrossRef]
89. Huang, S.; Huang, Z.; Chen, P.; Feng, C. Aberrant Chloride Intracellular Channel 4 Expression Is Associated With Adverse Outcome in Cytogenetically Normal Acute Myeloid Leukemia. *Front. Oncol.* **2020**, *10*, 1648. [CrossRef]
90. Skaar, J.R.; Pagan, J.K.; Pagano, M. SCF ubiquitin ligase-targeted therapies. *Nat. Rev. Drug Discov.* **2014**, *13*, 889–903. [CrossRef]
91. Liang, K.; Liao, L.; Liu, Q.; Ouyang, Q.; Jia, L.; Wu, G. microRNA-377-3p inhibits osteosarcoma progression by targeting CUL1 and regulating Wnt/β-catenin signaling pathway. *Clin. Transl. Oncol.* **2021**, *23*, 2350–2357. [CrossRef]

92. Lee, E.J.; Jo, M.; Rho, S.B.; Park, K.; Yoo, Y.N.; Park, J.; Chae, M.; Zhang, W.; Lee, J.H. Dkk3, downregulated in cervical cancer, functions as a negative regulator of β-catenin. *Int. J. Cancer* **2009**, *124*, 287–297. [CrossRef]
93. Katase, N.; Nagano, K.; Fujita, S. DKK3 expression and function in head and neck squamous cell carcinoma and other cancers. *J. Oral Biosci.* **2020**, *62*, 9–15. [CrossRef] [PubMed]
94. Moore, L.D.; Le, T.; Fan, G. DNA methylation and its basic function. *Neuropsychopharmacology* **2013**, *38*, 23–38. [CrossRef]
95. Liu, Y.; Cheng, H.; Cheng, C.; Zheng, F.; Zhao, Z.; Chen, Q.; Zeng, W.; Zhang, P.; Huang, C.; Jiang, W.; et al. ZNF191 alters DNA methylation and activates the PI3K-AKT pathway in hepatoma cells via transcriptional regulation of DNMT1. *Cancer Med.* **2022**, *11*, 1269–1280. [CrossRef] [PubMed]
96. Haque, S.; Vaiselbuh, S.R. Exosomal DNMT1 mRNA transcript is elevated in acute lymphoblastic leukemia which might reprograms leukemia progression. *Cancer Genet.* **2022**, *260–261*, 57–64. [CrossRef]
97. Yin, J.-Y.; Zhang, J.-T.; Zhang, W.; Zhou, H.-H.; Liu, Z.-Q. eIF3a: A new anticancer drug target in the eIF family. *Cancer Lett.* **2018**, *412*, 81–87. [CrossRef]
98. Chen, Y.-X.; Wang, C.-J.; Xiao, D.-S.; He, B.-M.; Li, M.; Yi, X.-P.; Zhang, W.; Yin, J.-Y.; Liu, Z.-Q. eIF3a R803K mutation mediates chemotherapy resistance by inducing cellular senescence in small cell lung cancer. *Pharmacol. Res.* **2021**, *174*, 105934. [CrossRef]
99. Zhu, X.N.; Wei, Y.S.; Yang, Q.; Liu, H.R.; Zhi, Z.; Zhu, D.; Xia, L.; Hong, D.L.; Yu, Y.; Chen, G.Q. FBXO22 promotes leukemogenesis by targeting BACH1 in MLL-rearranged acute myeloid leukemia. *J. Hematol. Oncol.* **2023**, *16*, 9. [CrossRef]
100. Greer, P. Closing in on the biological functions of Fps/Fes and Fer. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 278–289. [CrossRef] [PubMed]
101. Wei, C.; Wu, S.; Li, X.; Wang, Y.; Ren, R.; Lai, Y.; Ye, J. High expression of FER tyrosine kinase predicts poor prognosis in clear cell renal cell carcinoma. *Oncol. Lett.* **2013**, *5*, 473–478. [CrossRef]
102. Baldassarre, M.; Razinia, Z.; Burande, C.F.; Lamsoul, I.; Lutz, P.G.; Calderwood, D.A. Filamins regulate cell spreading and initiation of cell migration. *PLoS ONE* **2009**, *4*, e7830. [CrossRef]
103. del Valle-Pérez, B.; Martínez, V.G.; Lacasa-Salavert, C.; Figueras, A.; Shapiro, S.S.; Takafuta, T.; Casanovas, O.; Capellà, G.; Ventura, F.; Viñals, F. Filamin B plays a key role in vascular endothelial growth factor-induced endothelial cell motility through its interaction with Rac-1 and Vav-2. *J. Biol. Chem.* **2010**, *285*, 10748–10760. [CrossRef] [PubMed]
104. Chiu, D.S.; Oram, J.F.; LeBoeuf, R.C.; Alpers, C.E.; O'Brien, K.D. High-density lipoprotein-binding protein (HBP)/vigilin is expressed in human atherosclerotic lesions and colocalizes with apolipoprotein E. *Arterioscler. Thromb. Vasc. Biol.* **1997**, *17*, 2350–2358. [CrossRef]
105. Yang, W.L.; Wei, L.; Huang, W.Q.; Li, R.; Shen, W.Y.; Liu, J.Y.; Xu, J.M.; Li, B.; Qin, Y. Vigilin is overexpressed in hepatocellular carcinoma and is required for HCC cell proliferation and tumor growth. *Oncol. Rep.* **2014**, *31*, 2328–2334. [CrossRef] [PubMed]
106. Woo, H.-H.; Yi, X.; Lamb, T.; Menzl, I.; Baker, T.; Shapiro, D.J.; Chambers, S.K. Posttranscriptional suppression of proto-oncogene c-fms expression by vigilin in breast cancer. *Mol. Cell. Biol.* **2011**, *31*, 215–225. [CrossRef]
107. Han, S.P.; Tang, Y.H.; Smith, R. Functional diversity of the hnRNPs: Past, present and perspectives. *Biochem. J.* **2010**, *430*, 379–392. [CrossRef] [PubMed]
108. Sakuma, K.; Sasaki, E.; Kimura, K.; Komori, K.; Shimizu, Y.; Yatabe, Y.; Aoki, M. HNRNPLL stabilizes mRNA for DNA replication proteins and promotes cell cycle progression in colorectal cancer cells. *Cancer Sci.* **2018**, *109*, 2458–2468. [CrossRef]
109. Banerjee-Basu, S.; Baxevanis, A.D. Molecular evolution of the homeodomain family of transcription factors. *Nucleic Acids Res.* **2001**, *29*, 3258–3269. [CrossRef]
110. Zhang, Y.; Yu, Y.; Su, X.; Lu, Y. HOXD8 inhibits the proliferation and migration of triple-negative breast cancer cells and induces apoptosis in them through regulation of AKT/mTOR pathway. *Reprod. Biol.* **2021**, *21*, 100544. [CrossRef]
111. Laupitz, R.; Hecht, S.; Amslinger, S.; Zepeck, F.; Kaiser, J.; Richter, G.; Schramek, N.; Steinbacher, S.; Huber, R.; Arigoni, D. Biochemical characterization of Bacillus subtilis type II isopentenyl diphosphate isomerase, and phylogenetic distribution of isoprenoid biosynthesis pathways. *Eur. J. Biochem.* **2004**, *271*, 2658–2669. [CrossRef]
112. Marona, P.; Górka, J.; Mazurek, Z.; Wilk, W.; Rys, J.; Majka, M.; Jura, J.; Miekus, K. MCPIP1 Downregulation in Clear Cell Renal Cell Carcinoma Promotes Vascularization and Metastatic Progression. *Cancer Res.* **2017**, *77*, 4905–4920. [CrossRef]
113. Hirokawa, N.; Tanaka, Y. Kinesin superfamily proteins (KIFs): Various functions and their relevance for important phenomena in life and diseases. *Exp. Cell Res.* **2015**, *334*, 16–25. [CrossRef]
114. Su, M.; Guo, J.; Huang, J. Meta-analysis of the correlation between the rs17401966 polymorphism in kinesin family member 1B and susceptibility to hepatitis B virus related hepatocellular carcinoma. *Clin. Mol. Hepatol.* **2017**, *23*, 138–146. [CrossRef] [PubMed]
115. Akbari Moqadam, F.; Lange-Turenhout, E.A.M.; Ariës, I.M.; Pieters, R.; den Boer, M.L. MiR-125b, miR-100 and miR-99a co-regulate vincristine resistance in childhood acute lymphoblastic leukemia. *Leuk. Res.* **2013**, *37*, 1315–1321. [CrossRef] [PubMed]
116. Hansson, A.; Bloor, B.K.; Sarang, Z.; Haig, Y.; Morgan, P.R.; Stark, H.J.; Fusenig, N.E.; Ekstrand, J.; Grafström, R.C. Analysis of proliferation, apoptosis and keratin expression in cultured normal and immortalized human buccal keratinocytes. *Eur. J. Oral Sci.* **2003**, *111*, 34–41. [CrossRef] [PubMed]

117. Silveira, N.J.F.; Varuzza, L.; Machado-Lima, A.; Lauretto, M.S.; Pinheiro, D.G.; Rodrigues, R.V.; Severino, P.; Nobrega, F.G.; Silva, W.A.; de B Pereira, C.A.; et al. Searching for molecular markers in head and neck squamous cell carcinomas (HNSCC) by statistical and bioinformatic analysis of larynx-derived SAGE libraries. *BMC Med. Genom.* **2008**, *1*, 56. [CrossRef]
118. Karol, S.E.; Mattano, L.A., Jr.; Yang, W.; Maloney, K.W.; Smith, C.; Liu, C.; Ramsey, L.B.; Fernandez, C.A.; Chang, T.Y.; Neale, G. Genetic risk factors for the development of osteonecrosis in children under age 10 treated for acute lymphoblastic leukemia. *Blood J. Am. Soc. Hematol.* **2016**, *127*, 558–564. [CrossRef]
119. Maamari, D.; El-Khoury, H.; Saifi, O.; Muwakkit, S.A.; Zgheib, N.K. Implementation of Pharmacogenetics to Individualize Treatment Regimens for Children with Acute Lymphoblastic Leukemia. *Pharmacogenomics Pers. Med.* **2020**, *13*, 295–317. [CrossRef]
120. Ponting, C.P.; Oliver, P.L.; Reik, W. Evolution and functions of long noncoding RNAs. *Cell* **2009**, *136*, 629–641. [CrossRef]
121. Liu, Y.J.; Hounye, A.H.; Wang, Z.; Liu, X.; Yi, J.; Qi, M. Identification and validation of three autophagy-related long noncoding RNAs as prognostic signature in cholangiocarcinoma. *Front. Oncol.* **2021**, *11*, 780601. [CrossRef]
122. Yang, F.; Jing, F.; Li, Y.; Kong, S.; Zhang, S.; Huo, Y.; Huang, X.; Yu, S. Plasma lncRNA LOC338963 and mRNA AP3B2 are upregulated in paraneoplastic Lambert-Eaton myasthenic syndrome. *Muscle Nerve* **2022**, *66*, 216–222. [CrossRef]
123. Czabotar, P.E.; Lessene, G.; Strasser, A.; Adams, J.M. Control of apoptosis by the BCL-2 protein family: Implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 49–63. [CrossRef] [PubMed]
124. Wei, A.H.; Roberts, A.W.; Spencer, A.; Rosenberg, A.S.; Siegel, D.; Walter, R.B.; Caenepeel, S.; Hughes, P.; McIver, Z.; Mezzi, K.; et al. Targeting MCL-1 in hematologic malignancies: Rationale and progress. *Blood Rev.* **2020**, *44*, 100672. [CrossRef]
125. Lo, W.-K.; Biswas, S.K.; Brako, L.; Shiels, A.; Gu, S.; Jiang, J.X. Aquaporin-0 targets interlocking domains to control the integrity and transparency of the eye lens. *Investig. Ophthalmol. Vis. Sci.* **2014**, *55*, 1202–1212. [CrossRef]
126. Khan, S.; Ricciardelli, C.; Yool, A.J. Targeting aquaporins in novel therapies for male and female breast and reproductive cancers. *Cells* **2021**, *10*, 215. [CrossRef]
127. Larance, M.; Kirkwood, K.J.; Xirodimas, D.P.; Lundberg, E.; Uhlen, M.; Lamond, A.I. Characterization of MRFAP1 turnover and interactions downstream of the NEDD8 pathway. *Mol. Cell. Proteom.* **2012**, *11*, 014407. [CrossRef] [PubMed]
128. Hu, L.; Bai, Z.; Ma, X.; Bai, N.; Zhang, Z. MRFAP1 plays a protective role in neddylation inhibitor MLN4924-mediated gastric cancer cell death. *Eur. Rev. Med. Pharmacol. Sci.* **2018**, *22*, 8273–8280.
129. Vicente-Manzanares, M.; Zareno, J.; Whitmore, L.; Choi, C.K.; Horwitz, A.F. Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. *J. Cell Biol.* **2007**, *176*, 573–580. [CrossRef] [PubMed]
130. Jin, Q.; Cheng, M.; Xia, X.; Han, Y.; Zhang, J.; Cao, P.; Zhou, G. Down-regulation of MYH10 driven by chromosome 17p13.1 deletion promotes hepatocellular carcinoma metastasis through activation of the EGFR pathway. *J. Cell Mol. Med.* **2021**, *25*, 11142–11156. [CrossRef]
131. Milewicz, D.M.; Kwartler, C.S. Chapter 97—Genetic Variants in Smooth Muscle Contraction and Adhesion Genes Cause Thoracic Aortic Aneurysms and Dissections and Other Vascular Diseases. In *Muscle*; Hill, J.A., Olson, E.N., Eds.; Academic Press: Waltham, MA, USA, 2012; pp. 1291–1300. [CrossRef]
132. Wang, R.J.; Wu, P.; Cai, G.X.; Wang, Z.M.; Xu, Y.; Peng, J.J.; Sheng, W.Q.; Lu, H.F.; Cai, S.J. Down-regulated MYH11 expression correlates with poor prognosis in stage II and III colorectal cancer. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 7223–7228. [CrossRef]
133. Wang, J.; Xu, P.; Hao, Y.; Yu, T.; Liu, L.; Song, Y.; Li, Y. Interaction between DNMT3B and MYH11 via hypermethylation regulates gastric cancer progression. *BMC Cancer* **2021**, *21*, 914. [CrossRef]
134. Krendel, M.; Mooseker, M.S. Myosins: Tails (and heads) of functional diversity. *Physiology* **2005**, *20*, 239–251. [CrossRef] [PubMed]
135. Wang, Z.; Ying, M.; Wu, Q.; Wang, R.; Li, Y. Overexpression of myosin VI regulates gastric cancer cell progression. *Gene* **2016**, *593*, 100–109. [CrossRef] [PubMed]
136. Placzek, W.J.; Almeida, M.S.; Wüthrich, K. NMR structure and functional characterization of a human cancer-related nucleoside triphosphatase. *J. Mol. Biol.* **2007**, *367*, 788–801. [CrossRef]
137. Shang, H.; Zhang, H.; Ren, Z.; Zhao, H.; Zhang, Z.; Tong, J. Characterization of the Potential Role of NTPCR in Epithelial Ovarian Cancer by Integrating Transcriptomic and Metabolomic Analysis. *Front. Genet.* **2021**, *12*, 695245. [CrossRef]
138. Meissner, C.; Lorenz, H.; Weihofen, A.; Selkoe, D.J.; Lemberg, M.K. The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *J. Neurochem.* **2011**, *117*, 856–867. [CrossRef]
139. Qin, C.; Wang, Y.; Zhao, B.; Li, Z.; Li, T.; Yang, X.; Zhao, Y.; Wang, W. STOML2 restricts mitophagy and increases chemosensitivity in pancreatic cancer through stabilizing PARL-induced PINK1 degradation. *Cell Death Dis.* **2023**, *14*, 191. [CrossRef]
140. Du, Z.; Fei, T.; Verhaak, R.G.; Su, Z.; Zhang, Y.; Brown, M.; Chen, Y.; Liu, X.S. Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. *Nat. Struct. Mol. Biol.* **2013**, *20*, 908–913. [CrossRef] [PubMed]
141. Chen, Y.; Hong, C.; Qu, J.; Chen, J.; Qin, Z. Knockdown of lncRNA PCAT6 suppresses the growth of non-small cell lung cancer cells by inhibiting macrophages M2 polarization via miR-326/KLF1 axis. *Bioengineered* **2022**, *13*, 12834–12846. [CrossRef]
142. Schagdarsurengin, U.; Paradowska, A.; Steger, K. Analysing the sperm epigenome: Roles in early embryogenesis and assisted reproduction. *Nat. Rev. Urol.* **2012**, *9*, 609–619. [CrossRef]

143. Chen, Z.; Shi, C.; Gao, S.; Song, D.; Feng, Y. Impact of protamine I on colon cancer proliferation, invasion, migration, diagnosis and prognosis. *Biol. Chem.* **2018**, *399*, 265–275. [CrossRef]
144. Hendriks, W.J.; Pulido, R. Protein tyrosine phosphatase variants in human hereditary disorders and disease susceptibilities. *Biochim. Biophys. Acta (BBA)-Mol. Basis Dis.* **2013**, *1832*, 1673–1696. [CrossRef] [PubMed]
145. Karaca Atabay, E.; Mecca, C.; Wang, Q.; Ambrogio, C.; Mota, I.; Prokoph, N.; Mura, G.; Martinengo, C.; Patrucco, E.; Leonardi, G.; et al. Tyrosine phosphatases regulate resistance to ALK inhibitors in ALK+ anaplastic large cell lymphoma. *Blood* **2022**, *139*, 717–731. [CrossRef] [PubMed]
146. Zhang, S. Regulation of FYN Phosphorylation by the PTPN23 Tumor Suppressor Phosphatase in Breast Tumorigenesis. Ph.D. Thesis, State University of New York at Stony Brook, Stony Brook, NY, USA, 2017.
147. Wang, T.; Hong, W. Interorganellar regulation of lysosome positioning by the Golgi apparatus through Rab34 interaction with Rab-interacting lysosomal protein. *Mol. Biol. Cell* **2002**, *13*, 4317–4332. [CrossRef]
148. Wu, J.; Lu, Y.; Qin, A.; Qiao, Z.; Jiang, X. Overexpression of RAB34 correlates with poor prognosis and tumor progression in hepatocellular carcinoma. *Oncol. Rep.* **2017**, *38*, 2967–2974. [CrossRef]
149. Zeigerer, A.; Gilleron, J.; Bogorad, R.L.; Marsico, G.; Nonaka, H.; Seifert, S.; Epstein-Barash, H.; Kuchimanchi, S.; Peng, C.G.; Ruda, V.M. Rab5 is necessary for the biogenesis of the endolysosomal system in vivo. *Nature* **2012**, *485*, 465–470. [CrossRef] [PubMed]
150. Tan, Y.S.; Kim, M.; Kingsbury, T.J.; Civin, C.I.; Cheng, W.-C. Regulation of RAB5C is important for the growth inhibitory effects of MiR-509 in human precursor-B acute lymphoblastic leukemia. *PLoS ONE* **2014**, *9*, e111777. [CrossRef]
151. Julian, L.; Olson, M.F. Rho-associated coiled-coil containing kinases (ROCK) structure, regulation, and functions. *Small GTPases* **2014**, *5*, e29846. [CrossRef]
152. Warner, J.R.; McIntosh, K.B. How common are extraribosomal functions of ribosomal proteins? *Mol. Cell* **2009**, *34*, 3–11. [CrossRef]
153. Labriet, A.; Lévesque, É.; Cecchin, E.; De Mattia, E.; Villeneuve, L.; Rouleau, M.; Jonker, D.; Couture, F.; Simonyan, D.; Allain, E.P.; et al. Germline variability and tumor expression level of ribosomal protein gene RPL28 are associated with survival of metastatic colorectal cancer patients. *Sci. Rep.* **2019**, *9*, 13008. [CrossRef]
154. Usami, Y.; Wu, Y.; Göttlinger, H.G. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. *Nature* **2015**, *526*, 218–223. [CrossRef]
155. Terasawa, K.; Sagae, S.; Toyota, M.; Tsukada, K.; Ogi, K.; Satoh, A.; Mita, H.; Imai, K.; Tokino, T.; Kudo, R. Epigenetic Inactivation of TMS1/ASC in Ovarian Cancer. *Clin. Cancer Res.* **2004**, *10*, 2000–2006. [CrossRef]
156. Konyukh, M.; Delorme, R.; Chaste, P.; Leblond, C.; Lemière, N.; Nygren, G.; Anckarsäter, H.; Rastam, M.; Ståhlberg, O.; Amsellem, F.; et al. Variations of the candidate SEZ6L2 gene on Chromosome 16p11.2 in patients with autism spectrum disorders and in human populations. *PLoS ONE* **2011**, *6*, e17289. [CrossRef]
157. Chen, L.; Han, S.; Li, Y.; Zheng, Y.; Zhang, Q. SEZ6L2, regulated by USF1, accelerates the growth and metastasis of breast cancer. *Exp. Cell Res.* **2022**, *417*, 113194. [CrossRef]
158. Walz, A.; Ooms, A.; Gadd, S.; Gerhard, D.; Smith, M.; GuidryáAuvil, J.M.; Meerzaman, D.; Chen, Q.-R.; Hsu, C.; Yan, C. Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. *Cancer Cell* **2015**, *27*, 286–297. [CrossRef]
159. Wan, Z.H.; Ma, Y.H.; Jiang, T.Y.; Lin, Y.K.; Shi, Y.Y.; Tan, Y.X.; Dong, L.W.; Wang, H.Y. Six2 is negatively correlated with prognosis and facilitates epithelial-mesenchymal transition via TGF-β/Smad signal pathway in hepatocellular carcinoma. *Hepatobiliary Pancreat. Dis. Int.* **2019**, *18*, 525–531. [CrossRef]
160. Li, P.; Noegel, A.A. Inner nuclear envelope protein SUN1 plays a prominent role in mammalian mRNA export. *Nucleic Acids Res.* **2015**, *43*, 9874–9888. [CrossRef]
161. Liu, L.; Li, S.W.; Yuan, W.; Tang, J.; Sang, Y. Downregulation of SUN2 promotes metastasis of colon cancer by activating BDNF/TrkB signalling by interacting with SIRT1. *J. Pathol.* **2021**, *254*, 531–542. [CrossRef]
162. Lennard, A.; Gaston, K.; Fried, M. The Surf-1 and Surf-2 genes and their essential bidirectional promoter elements are conserved between mouse and human. *DNA Cell Biol.* **1994**, *13*, 1117–1126. [CrossRef]
163. Jung, C.H.; Kim, E.M.; Song, J.Y.; Park, J.K.; Um, H.D. Mitochondrial superoxide dismutase 2 mediates γ-irradiation-induced cancer cell invasion. *Exp. Mol. Med.* **2019**, *51*, 1–10. [CrossRef]
164. Hatzis, P.; van der Flier, L.G.; van Driel, M.A.; Guryev, V.; Nielsen, F.; Denissov, S.; Nijman, I.c.J.; Koster, J.; Santo, E.E.; Welboren, W. Genome-wide pattern of TCF7L2/TCF4 chromatin occupancy in colorectal cancer cells. *Mol. Cell. Biol.* **2008**, *28*, 2732–2744. [CrossRef]
165. Desterke, C.; Hugues, P.; Hwang, J.W.; Bennaceur-Griscelli, A.; Turhan, A.G. Embryonic Program Activated during Blast Crisis of Chronic Myelogenous Leukemia (CML) Implicates a TCF7L2 and MYC Cooperative Chromatin Binding. *Int. J. Mol. Sci.* **2020**, *21*, 4057. [CrossRef]
166. Egeblad, M.; Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer* **2002**, *2*, 161–174. [CrossRef]

167. Saleh, M.; Khalil, M.; Abdellateif, M.S.; Ebeid, E.; Madney, Y.; Kandeel, E.Z. Role of matrix metalloproteinase MMP-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP-1) in the clinical progression of pediatric acute lymphoblastic leukemia. *Hematology* **2021**, *26*, 758–768. [CrossRef]
168. Yang, W.B.; Hao, F.; Song, Z.Q.; Yang, X.C.; Ni, B. Apoptosis of the dermal papilla cells of hair follicle associated with the expression of gene HSPCO16 in vitro. *Exp. Dermatol.* **2005**, *14*, 209–214. [CrossRef]
169. Song, Z.; Zhou, C.; Wang, J.; Yang, W.; Hao, F. Effect of HSPC016 gene expression on the aggregative growth of dermal papillae cells. *Australas. J. Dermatol.* **2012**, *53*, e26–e29. [CrossRef]
170. Van Everdink, W.; Baranova, A.; Lummen, C.; Tyazhelova, T.; Looman, M.; Ivanov, D.; Verlind, E.; Pestova, A.; Faber, H.; van der Veen, A. RFP2, c13ORF1, and FAM10A4 are the most likely tumor suppressor gene candidates for B-cell chronic lymphocytic leukemia. *Cancer Genet. Cytogenet.* **2003**, *146*, 48–57. [CrossRef]
171. Dai, T.; Ye, L.; Deng, M.; Lin, G.; Liu, R.; Yu, H.; Liu, W.; Yang, Y.; Wang, G. Upregulation of TMCO3 Promoting Tumor Progression and Contributing to the Poor Prognosis of Hepatocellular Carcinoma. *J. Clin. Transl. Hepatol.* **2022**, *10*, 913–924. [CrossRef]
172. Taylor, M.R.; Slavov, D.; Gajewski, A.; Vlcek, S.; Ku, L.; Fain, P.R.; Carniel, E.; Di Lenarda, A.; Sinagra, G.; Boucek, M.M. Thymopoietin (lamina-associated polypeptide 2) gene mutation associated with dilated cardiomyopathy. *Human. Mutat.* **2005**, *26*, 566–574. [CrossRef]
173. Zhang, L.; Wang, G.; Chen, S.; Ding, J.; Ju, S.; Cao, H.; Tian, H. Depletion of thymopoietin inhibits proliferation and induces cell cycle arrest/apoptosis in glioblastoma cells. *World J. Surg. Oncol.* **2016**, *14*, 267. [CrossRef]
174. Chacinska, A.; Koehler, C.M.; Milenkovic, D.; Lithgow, T.; Pfanner, N. Importing mitochondrial proteins: Machineries and mechanisms. *Cell* **2009**, *138*, 628–644. [CrossRef]
175. Park, S.H.; Lee, A.R.; Choi, K.; Joung, S.; Yoon, J.B.; Kim, S. TOMM20 as a potential therapeutic target of colorectal cancer. *BMB Rep.* **2019**, *52*, 712–717. [CrossRef] [PubMed]
176. De Paula, A.M.; Franques, J.; Fernandez, C.; Monnier, N.; Lunardi, J.; Pellissier, J.-F.; Figarella-Branger, D.; Pouget, J. A TPM3 mutation causing cap myopathy. *Neuromuscul. Disord.* **2009**, *19*, 685–688. [CrossRef] [PubMed]
177. Xu, X.; Wang, Y.; Bryce, N.S.; Tang, K.; Meagher, N.S.; Kang, E.Y.; Kelemen, L.E.; Köbel, M.; Ramus, S.J.; Friedlander, M. Targeting the actin/tropomyosin cytoskeleton in epithelial ovarian cancer reveals multiple mechanisms of synergy with anti-microtubule agents. *Br. J. Cancer* **2021**, *125*, 265–276. [CrossRef] [PubMed]
178. Zhu, F.; Yan, P.; Zhang, J.; Cui, Y.; Zheng, M.; Cheng, Y.; Guo, Y.; Yang, X.; Guo, X.; Zhu, H. Deficiency of TPPP2, a factor linked to oligoasthenozoospermia, causes subfertility in male mice. *J. Cell Mol. Med.* **2019**, *23*, 2583–2594. [CrossRef]
179. Xu, Y.; Xu, X.; Ni, X.; Pan, J.; Chen, M.; Lin, Y.; Zhao, Z.; Zhang, L.; Ge, N.; Song, G. Gene-based cancer-testis antigens as prognostic indicators in hepatocellular carcinoma. *Heliyon* **2023**, *9*, e13269. [CrossRef]
180. Rho, S.B.; Lee, J.H.; Park, M.S.; Byun, H.-J.; Kang, S.; Seo, S.-S.; Kim, J.-Y.; Park, S.-Y. Anti-apoptotic protein TCTP controls the stability of the tumor suppressor p53. *FEBS Lett.* **2011**, *585*, 29–35. [CrossRef]
181. He, S.; Huang, Y.; Wang, Y.; Tang, J.; Song, Y.; Yu, X.; Ma, J.; Wang, S.; Yin, H.; Li, Q. Histamine-releasing factor/translationally controlled tumor protein plays a role in induced cell adhesion, apoptosis resistance and chemoresistance in non-Hodgkin lymphomas. *Leuk. Lymphoma* **2015**, *56*, 2153–2161. [CrossRef]
182. Kang, B.H.; Plescia, J.; Dohi, T.; Rosa, J.; Doxsey, S.J.; Altieri, D.C. Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. *Cell* **2007**, *131*, 257–270. [CrossRef]
183. Chen, R.; Pan, S.; Lai, K.; Lai, L.A.; Crispin, D.A.; Bronner, M.P.; Brentnall, T.A. Up-regulation of mitochondrial chaperone TRAP1 in ulcerative colitis associated colorectal cancer. *World J. Gastroenterol.* **2014**, *20*, 17037–17048. [CrossRef]
184. Ariës, I.M.; Bodaar, K.; Karim, S.A.; Chonghaile, T.N.; Hinze, L.; Burns, M.A.; Pfirrmann, M.; Degar, J.; Landrigan, J.T.; Balbach, S.; et al. PRC2 loss induces chemoresistance by repressing apoptosis in T cell acute lymphoblastic leukemia. *J. Exp. Med.* **2018**, *215*, 3094–3114. [CrossRef]
185. Jordan, M. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. *Curr. Med. Chem.-Anti-Cancer Agents* **2002**, *2*, 1–17. [CrossRef]
186. Nami, B.; Wang, Z. Genetics and expression profile of the tubulin gene superfamily in breast cancer subtypes and its relation to taxane resistance. *Cancers* **2018**, *10*, 274. [CrossRef] [PubMed]
187. Djureinovic, D.; Hallström, B.M.; Horie, M.; Mattsson, J.S.M.; La Fleur, L.; Fagerberg, L.; Brunnström, H.; Lindskog, C.; Madjar, K.; Rahnenführer, J.; et al. Profiling cancer testis antigens in non-small-cell lung cancer. *JCI Insight* **2016**, *1*, e86837. [CrossRef] [PubMed]
188. Marinovic, A.C.; Mitch, W.E.; Price, S.R. Tools for evaluating ubiquitin (UbC) gene expression: Characterization of the rat UbC promoter and use of an unique 3′ mRNA sequence. *Biochem. Biophys. Res. Commun.* **2000**, *274*, 537–541. [CrossRef]
189. Kim, J.; Kim, Y.; Choi, H.; Kwon, A.; Jekarl, D.W.; Lee, S.; Jang, W.; Chae, H.; Kim, J.R.; Kim, J.M.; et al. Ubiquitin C decrement plays a pivotal role in replicative senescence of bone marrow mesenchymal stromal cells. *Cell Death Dis.* **2018**, *9*, 139. [CrossRef] [PubMed]

190. Alchahin, A.M.; Mei, S.; Tsea, I.; Hirz, T.; Kfoury, Y.; Dahl, D.; Wu, C.-L.; Subtelny, A.O.; Wu, S.; Scadden, D.T. A transcriptional metastatic signature predicts survival in clear cell renal cell carcinoma. *Nat. Commun.* **2022**, *13*, 1–15. [CrossRef]
191. Metzger, M.B.; Hristova, V.A.; Weissman, A.M. HECT and RING finger families of E3 ubiquitin ligases at a glance. *J. Cell science* **2012**, *125*, 531–537. [CrossRef]
192. Hu, K.; Liu, X.; Li, Y.; Li, Q.; Xu, Y.; Zeng, W.; Zhong, G.; Yu, C. Exosomes Mediated Transfer of Circ_UBE2D2 Enhances the Resistance of Breast Cancer to Tamoxifen by Binding to MiR-200a-3p. *Med. Sci. Monit.* **2020**, *26*, e922253. [CrossRef]
193. Wen, J.L.; Wen, X.F.; Li, R.B.; Jin, Y.C.; Wang, X.L.; Zhou, L.; Chen, H.X. UBE3C promotes growth and metastasis of renal cell carcinoma via activating Wnt/β-catenin pathway. *PLoS ONE* **2015**, *10*, e0115622. [CrossRef]
194. Huang, L.Z.; Li, Y.J.; Xie, X.F.; Zhang, J.J.; Cheng, C.Y.; Yamashiro, K.; Chen, L.J.; Ma, X.Y.; Cheung, C.M.; Wang, Y.S.; et al. Whole-exome sequencing implicates UBE3D in age-related macular degeneration in East Asian populations. *Nat. Commun.* **2015**, *6*, 6687. [CrossRef]
195. Liu, H.; Heller-Trulli, D.; Moore, C.L. Targeting the mRNA endonuclease CPSF73 inhibits breast cancer cell migration, invasion, and self-renewal. *iScience* **2022**, *25*, 104804. [CrossRef]
196. Kimura, K.; Wakamatsu, A.; Suzuki, Y.; Ota, T.; Nishikawa, T.; Yamashita, R.; Yamamoto, J.; Sekine, M.; Tsuritani, K.; Wakaguri, H.; et al. Diversification of transcriptional modulation: Large-scale identification and characterization of putative alternative promoters of human genes. *Genome Res.* **2006**, *16*, 55–65. [CrossRef] [PubMed]
197. Yang, C.; Wu, S.; Wu, X.; Zhou, X.; Jin, S.; Jiang, H. Silencing circular RNA UVRAG inhibits bladder cancer growth and metastasis by targeting the microRNA-223/fibroblast growth factor receptor 2 axis. *Cancer Sci.* **2019**, *110*, 99–106. [CrossRef] [PubMed]
198. Bänziger, C.; Soldini, D.; Schütt, C.; Zipperlen, P.; Hausmann, G.; Basler, K. Wntless, a conserved membrane protein dedicated to the secretion of Wnt proteins from signaling cells. *Cell* **2006**, *125*, 509–522. [CrossRef]
199. Zhang, W.; Tao, H.; Chen, X.; Sugimura, H.; Wang, J.; Zhou, P. High expression of Wls is associated with lymph node metastasis and advanced TNM stage in gastric carcinomas. *Pathol. Int.* **2017**, *67*, 141–146. [CrossRef]
200. Chiou, S.-S.; Wang, L.-T.; Huang, S.-B.; Chai, C.-Y.; Wang, S.-N.; Liao, Y.-M.; Lin, P.-C.; Liu, K.-Y.; Hsu, S.-H. Wntless (GPR177) expression correlates with poor prognosis in B-cell precursor acute lymphoblastic leukemia via Wnt signaling. *Carcinogenesis* **2014**, *35*, 2357–2364. [CrossRef]
201. Memarian, A.; Vosough, P.; Asgarian-Omran, H.; Tabrizi, M.; Shabani, M.; Shokri, F. Differential WNT gene expression in various subtypes of acute lymphoblastic leukemia. *Iran. J. Immunol.* **2012**, *9*, 61–71.
202. Mangino, M.; Hwang, S.-J.; Spector, T.D.; Hunt, S.C.; Kimura, M.; Fitzpatrick, A.L.; Christiansen, L.; Petersen, I.; Elbers, C.C.; Harris, T. Genome-wide meta-analysis points to CTC1 and ZNF676 as genes regulating telomere homeostasis in humans. *Human Mol. Genet.* **2012**, *21*, 5385–5394. [CrossRef]
203. Peculis, R.; Niedra, H.; Rovite, V. Large Scale Molecular Studies of Pituitary Neuroendocrine Tumors: Novel Markers, Mechanisms and Translational Perspectives. *Cancers* **2021**, *13*, 1395. [CrossRef]