EGFR mutations are associated with response to depatux-m in combination with temozolomide and result in a receptor that is hypersensitive to ligand

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Abstract: Background: The randomized phase II INTELLANCE-2/EORTC 1410 trial on EGFR−amplified recurrent glioblastoma temozolomide compared with the control arm of alkylating chemotherapy only. We here performed translational research. Targeted DNA−sequencing and whole transcriptome analysis was performed on clinical trial samples. High-throughput, high-content imaging analysis was done to understand the molecular mechanism underlying the survival benefit. We first defined the tumor genomic landscape in this well−annotated patient population. We find that tumors harboring EGFR nucleotide variations (SNVs) have improved outcome in the depatux−m + TMZ combination arm. Such SNVs are commonly low affinity EGFR ligands. These hypersensitizing SNVs and ligand−independent EGFR V1171Y variant are inversely correlated. Increased ligand−induced activation will result in increased exposure of the epitope to the antibody−drug conjugate. Weaker kinase inhibitors, providing a potential alternative treatment strategy. Conclusions: These data can help guide treatment for EGFR mutation−positive glioblastoma.

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Y. Hoogstrate¹, W. Vallentgoed¹, J.M. Kros³, I.de Heer¹, M. de Wit¹, M. Eoli⁴, J. Sepulveda⁵, A. Walenkamp⁶, J-S Frenel⁷, E. Franceschi⁸, P.M. Clement⁹, M. Weller¹⁰, M. van Royen³,¹¹, P. Ansell¹², J. Looman¹², E. Bain¹², M. Morfouace¹³, T. Gorlia¹³, V. Golfinopoulos¹³, M. van den Bent¹, P. J. French¹,¹¹*

¹dept Neurology, ²Urology, ³Pathology and ¹¹Cancer Treatment Screening Facility, Erasmus MC, Rotterdam, Netherlands; ⁴Carlo Besta, Milano, Italy; ⁵12 Octubre Hospital, Madrid, Spain; ⁶UMCG, Groningen, the Netherlands; ⁷Gauducheau, Nantes, France; ⁸AUSL/IRCCS Institute of Neurological Sciences, Bologna, Italy; ⁹Leuven Cancer Institute, KU Leuven, Leuven, Belgium; ¹⁰Department of Neurology, University Hospital and University of Zurich, Switzerland; ¹²AbbVie, North Chicago, IL, United States, ¹³EORTC Headquarters, Brussels, Belgium.

Correspondance to:

Pim J. French,
Dept Neurology
Erasmus MC
POBox 2040
3000CA, Rotterdam
The Netherlands
P: +31 10 70 44333
M: p.french@erasusmc.nl

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Abstract

Background: The randomized phase II INTELLANCE-2/EORTC_1410 trial on EGFR-amplified recurrent glioblastomas showed a trend towards improved overall survival when patients were treated with depatux-m plus temozolomide compared with the control arm of alkylating chemotherapy only. We here performed translational research on material derived from this clinical trial to identify patients that benefit from this treatment.

Methods: Targeted DNA-sequencing and whole transcriptome analysis was performed on clinical trial samples. High-throughput, high-content imaging analysis was done to understand the molecular mechanism underlying the survival benefit.

Results: We first define the tumor genomic landscape in this well-annotated patient population. We find that tumors harbouring EGFR single nucleotide variations (SNVs) have improved outcome in the depatux-m + TMZ combination arm. Such SNVs are common to the extracellular domain of the receptor and functionally result in a receptor that is hypersensitive to low-affinity EGFR ligands. These hypersensitising SNVs and the ligand-independent EGFRvIII variant are inversely correlated, indicating two distinct modes of evolution to increase EGFR signalling in glioblastomas. Ligand-hypersensitivity can explain the therapeutic efficacy of depatux-m as increased ligand-induced activation will result in increased exposure of the epitope to the antibody-drug conjugate. We also identified tumors harbouring mutations sensitive to ‘classical’ EGFR tyrosine-kinase-inhibitors, providing a potential alternative treatment strategy.

Conclusions: These data can help guide treatment for recurrent glioblastoma patients and increase our understanding into the molecular mechanisms underlying EGFR-signalling in these tumors.

Keywords: Depatux-m; EGFR; amphiregulin; extracellular domain mutations; ligand hypersensitivity
Importance of the Study

Recurrent glioblastoma patients have a dismal prognosis; the median survival is ~6-8 months and there is no standard of care. Depatux-m is an antibody-drug conjugate with signs of clinical activity in recurrent glioblastomas when given in combination with temozolomide. Here, we used material from a randomized phase II trial and identified patients that have survival benefit from this combination.

We show that specific mutations increase sensitivity to EGFR ligands and this ‘hypersensitivity’ can explain the observed treatment benefit. Our results indicate that ligand-hypersensitivity and ligand-independence are two, inversely correlated, mechanisms to increase EGFR-signalling. We also identified tumors harbouring mutations sensitive to ‘classical’ EGFR tyrosine-kinase-inhibitors, providing a potential alternative treatment strategy. These data can help guide treatment for recurrent glioblastoma patients.

Keypoints

1. SNVs in EGFR are correlated with improved survival to depatux-m + temozolomide
2. Common SNVs in EGFR increase sensitivity of the receptor to its ligands.
3. Some gliomas harbour EGFR tyrosine kinase inhibitor sensitive mutations
Introduction

The epidermal growth factor receptor (EGFR) gene is amplified in approximately half of all glioblastoma patients\textsuperscript{1,2}. Unfortunately, and despite EGFR being a driver mutation in glioblastomas, pharmacological inhibition of the receptor has not been demonstrated to affect patient survival or tumor growth\textsuperscript{3-5}. Depatuxizumab mafodotin (depatux-m, ABT414), is an antibody–drug conjugate that consists of an antibody directed against EGFR\textsuperscript{6,7}, conjugated to a toxin (monomethyl auristatin F) that blocks microtubule polymerization. The antibody is specific for tumors that overexpress EGFR and preferentially binds to the active conformation of the receptor and the constitutively active variant EGFRvIII\textsuperscript{6-8}. Depatux-m therefore should specifically target glioblastoma cells by using the high expression level of EGFR. Phase I clinical trials have tested the drug for safety and toxicity, and showed some encouraging responses especially in recurrent glioblastoma patients with EGFR amplification\textsuperscript{9-11}.

INTELLANCE-2/EORTC_1410 was a randomized phase II trial on EGFR-amplified recurrent glioblastomas that showed a trend towards improved overall survival (HR 0.71, 95% CI [0.50, 1.02], p=0.06 in the primary analysis, HR 0.66, 95% CI [0.48, 0.93], p=0.024 in follow-up analysis) when patients were treated with depatux-m and temozolomide (TMZ) compared with the control arm of alkylating chemotherapy only. In the present study, we aimed to identify patients that benefit from this combination and to understand the mechanism of increased sensitivity.
Methods:

Patient samples

Recurrent GBM patients were considered eligible for the INTELLANCE-2/EORTC_1410 trial (NCT02343406) if they had been diagnosed with a histologically confirmed, \textit{EGFR-amplified} glioblastoma at first occurrence. Amplification of the EGFR locus was centrally determined using FISH in one of three laboratories (Histogenex, Antwerp Belgium, Mosaic, Lake Forest California, Peter MacCallum Cancer Institute (Melbourne Australia) using the Vysis EGFR CDx Assay (Abbott Molecular, Des Plaines, IL, USA; not on market)\textsuperscript{12}. A tumor was considered EGFR-amplified when the EGFR/centromere chromosome 7 (CEP7) ratio was $\geq 2$ in $\geq 15\%$ recorded nuclei, with 50 nuclei/tumor analyzed. Tumors with polysomy for chromosome 7 (CEP7 copy number $> 3$) but without focal amplification of the EGFR gene in $\geq 15\%$ nuclei were considered to be EGFR-nonamplified and not included. 260 patients were randomized in the trial to receive either i) TMZ or, if progressing within 16 weeks of day 1 of the last temozolomide cycle, CCNU (n=26 and n=60 respectively); ii) depatux-m (n=86) or; iii) TMZ plus depatux-m (n=88). For this analysis, the database was locked on 12\textsuperscript{th} of January 2018 (longer term follow-up data). \textit{MGMT} promoter methylation status data were previously described and determined using a methylation-specific PCR\textsuperscript{13}. All patients gave written informed consent for trial participation, pathology review and molecular testing.

Sequencing

Material, either tissue sections or tissue blocks were centrally collected at Erasmus MC. Evaluation of the area with highest tumor content was done by the pathologist (JMK) on a hematoxylin and eosin stained section. One to twenty $5\mu$ sections were then sent to Almac Diagnostics (Craigavon, UK) for macro-dissection, DNA and RNA extraction and sequencing. DNA/RNA extraction was performed using the Allprep DNA/RNA FFPE kit (Qiagen, Venlo, the Netherlands). Sequencing was done on the Trusight Tumor 170 panel (Illumina, Eindhoven, the Netherlands) which uses a combination of DNA and RNA sequencing to interrogate SNVs in ~150 genes, amplification of 59 genes, and fusion and splice-variant
expression in 55 genes. SNV, copy-number, fusion-gene and splice-variant expression calling was done using the Illumina Basespace sequence hub. Very deep sequencing was performed to enable quantification of subclonal \textit{EGFR} variants. All variants with a variant allele fraction (VAF) $> 15\%$ were included in the analysis, except for \textit{EGFR}, where all VAFs were included as all variants in \textit{EGFR} are subclonal. SNVs with quality scores $<70$ and/or present in the Exac database at fractions $>0.001$ were omitted from the analysis. Splice variants/mutations were calculated as the ‘spliced-in fraction’; the number of mutant reads as fraction of the total reads over that particular variant. Data were further analyzed in R using \textit{ggplot2}, \textit{survival} and \textit{GenVisR} packages. Expression values were estimated using \textit{featureCounts} using \textit{gencode-29} as gene annotation. One sample yielding only 707 reads was excluded from further analysis.

Whole transcriptome sequencing of rRNA depleted cDNA was done on the same isolate by GenomeScan (Leiden, the Netherlands) at a depth of 50 million paired-end reads per sample. \textit{HTStream} was used to remove duplicate reads, \textit{fastp} for low base quality trimming and adaptor removal and further quality assessment. Alignment to \textit{hg38} was done using \textit{STAR} (2.6.1d). Stranded read-counts were estimated with STARs builtin ‘--quantMode GeneCounts’ option. Samples with read-count $<750,000$ were excluded from further analysis (29 samples). \textit{DESeq2} was used for expression analysis and its VST-normalization for survival analysis using Coxph regression in R.

\textit{Data analysis}

For generating the waterfall plot of chromosomal changes, we defined trisomy as whole chromosome copy-number $> 2.4$ and LOH as copy-number $< 1.6$. For generating the waterfall plot per gene we set a threshold for high-copy amplification to $> 6$ copies per cell, copy number gain (including trisomy) between 3 and 6, and deep (homozygous) deletions at $< 1$ copy per cell. All analyses to define variants associated with survival were done on samples with high copy amplification of \textit{EGFR} only; samples without such high-level amplification may represent a different molecular entity.
Constructs and image analysis

EGFR mutation constructs were generated by in-fusion cloning into a piggybac vector (System Biosciences, Palo Alto, Ca) with eGFP cloned 3’ to the transmembrane domain as described \(^{14}\). This position was chosen to avoid potential interference with ligand binding or receptor internalization signaling sites. These constructs retain the important physical properties of EGFR with respect to signal transduction and protein-protein interactions\(^{14}\). Stable HeLa cell-lines (ATCC, Manassas, Virginia) were created for all constructs. Cells were plated in 96 or 384 well plates for further analysis. Following transfection, we selected for cells that expressed the (mutant) EGF receptor using FACS. As we did not select for a single cell clone, levels of EGFR expression was variable between individual cells. This way the observed responses can be evaluated across a wide range of expression levels. Quantification of EGFR signals intensities shows a high correlation between intensity and mRNA expression levels. This was done by comparing mRNA expression with signal intensities in various lung-cancer cell lines (manuscript in preparation). Using this approach we show that the various mutation constructs had expression levels comparable to the endogenous EGFR expression in these cells (except for the cell lines expressing the EGFR_A289D or EGFRvIII mutation, where the construct is expressed at slightly lower levels; for EGFRvIII this was despite repeated attempts). Moreover, expression levels were highly similar between the various mutation constructs, again except for the cell line expressing the EGFR_A289D or EGFRvIII mutation).

All images were obtained using an Opera Phenix high-throughput high-content confocal microscope (Perkin Elmer, Hamburg, Germany). At least 10 images were obtained per well so that hundreds of individual cells per condition were analysed ensuring robustness of measurements. Image analysis was performed using Harmony software (Perkin Elmer) using identical settings for all conditions within each experiment and further analysed using R. Experiments were performed at least in three independent replicates.

EGFR antibody (clone H11, DAKO, Amstelveen, the Netherlands) and a phospho-specific EGFR antibody (AB32430, anti phospho Y1068, Abcam, Cambridge, UK) were used at 1:500 dilution.
Secondary antibodies used were alexafluor 647 goat anti-mouse, alexafluor 594 rabbit anti-mouse and alexafluor 488 goat anti-rabbit (A21240, A11062 and A11008 respectively, Invitrogen, Bleiswijk, the Netherlands). Hoechst was used as counterstain to visualize nuclei.

**Statistical analysis**

Distribution of frequencies were compared between subtypes using the Chi-squared test. A Fisher’s exact test was used in case the assumptions for chi-square distribution were violated as indicated in the respective tables. Kaplan–Meier survival curves were generated using the survival package in R\(^1\). Overall survival was used to identify the molecular markers associated with outcome, with survival defined from the point of randomization until the date of death. If unavailable, the date of day known to be alive was used. The significance of prognostic factors was determined using Cox regression in univariate analysis. Differential gene expression was determined using de DESeq2 bioconductor package. P values less than 0.05, which were adjusted for a false discovery rate < 0.05, were considered significant.

**Results:**

**Mutational landscape of INTELLANCE-2/EORTC_1410 trial samples**

We first analysed the molecular characteristics of tumors from patients included in the INTELLANCE-2/EORTC_1410 trial. This is important as this allows defining the genomic landscape in tumors of patients that are eligible for clinical trial inclusion. Glioblastomas often have trisomy of chromosome 7 and loss of chromosome 10 and, of the large scale genomic changes, these were indeed the two most commonly found (204 and 184 samples for chromosome 7 and 10 respectively, figure 1a). Combined trisomy 7, LOH 10 was observed in 176 of 236 samples (75%). Other common large chromosomal changes included gain of chromosomes 19 and 20 which are also frequently observed in glioblastomas.
On the gene level, most, but not all, samples harboured high copy amplification of the **EGFR** locus (copy number > 6 in 200 tumors\(^\text{16}\)); this was mainly observed in tumors with trisomy 7 (166/200, figure 1b). Other high copy amplified genes included **MDM2** (n=20), **MDM4** (n=21), **CDK4** (n=24) and **CDK6** (n=4). Most samples also harboured a homozygous deletion of the **CDKN2A** locus, and a small population had such deletions in the **PTEN** locus.

Mutations were identified in driver genes common to GBMs and included **EGFR** (n=115), **PTEN** (n=62), **TP53** (n=48), **CREBBP** (n=21), and **PIK3CA** (n=16) (figure 1c). Mutational hotspots were identified in **TP53** (supplementary figure 1), **PTEN** (see below) and **EGFR**. As may be expected, truncating mutations were common to tumor suppressor genes (**PTEN, RB1** and **NF1**).

Common missense mutations identified in **EGFR** clustered on the extracellular domain of the protein and included R108, A289 and G598. Interestingly, we identified a new hotspot at the 3' (intracellular) end of the gene where truncating mutations tended to cluster (figure 2a). Such mutations often involved amino acids L1001 and M1002. We also identified three mutations in EGFR that, in lung cancer, are associated with response to EGFR tyrosine kinase inhibitors (TKIs)\(^\text{17}\). These mutations, G719A, G719D and S786I (~1.3% of analysed samples), were present at a relatively high mutant allele fraction (in two samples, the variant allele fraction, VAF, exceeded >20%). Since the type of mutation may predict response to EGFR tyrosine kinase inhibitors, such inhibitors may provide a new treatment option for these patients\(^\text{18-20}\).

The most common (oncogenic) splice variant identified was the glioblastoma-specific in-frame deletion of exons 2-7 (**EGFRvIII**), present in approximately half of all **EGFR**-amplified samples (n=101, figure 2b). Other variants present at significant population frequency included deletions of exons 9-10 (n=14), exons 25-26 (n=14) and exons 25-27 (n=22), the latter two affecting the intracellular domain. **EGFR** fusion genes were identified in 13 samples, with fusion partners often located in the vicinity of the **EGFR** gene locus (**SEPT14, SEC61G, LANCL2**). Similar to previously reported for glioblastomas, the mutations, splice variants and fusion genes identified in **EGFR** were almost always
subclonal and many samples harboured more than one genetic change (figure 2b). Only 13/200 EGFR-amplified samples did not harbour any genetic change in the EGFR locus.

**A289 missense mutations and 3’ truncating mutations in EGFR are associated with response to depatux-m + TMZ**

We performed correlative analysis on genetic changes to identify those associated with survival in the depatux-m + TMZ arm. Interestingly, the presence of SNVs (any protein altering SNV within the coding region) in EGFR was associated with survival in univariate analysis: the HR was 0.495 with 95% CI [0.283, 0.865], p = 0.014 in the combination arm and 0.751, 95%CI [0.444, 1.272], p = 0.287 in the depatux-m monotherapy arm, compared to the CCNU or TMZ control arm (table 1, figure 3). Multivariable analysis confirmed that depatux-m + TMZ treatment was associated with survival in samples with EGFR-SNVs, independent of known prognostic factors such as age and MGMT promoter methylation status (HR 0.45, 95% CI[0.26, 0.76], p = 0.003, supplementary table 1). No such association was found in the samples without EGFR-SNVs (HR 0.85, 95% CI[0.48, 1.50], p = 0.57). Since depatux-m specifically targets EGFR, we focussed further on individual variants to determine which of these were most associated with survival. A trend was observed in tumors harbouring A289 hotspot mutations (HR 0.386, 95% CI[0.138, 1.082], p=0.070, table 1, figure 4a). No other individual SNV reached such statistical values, but this may be related to the low number of samples harbouring individual SNVs and the corresponding limited statistical power.

On the splice variant level, we found that a deletion of exons 25-27, affecting the C-terminal intracellular domain, was also associated with survival in the depatux-m + TMZ arm. Although there are relatively few samples (n=22) with this genetic change, the HR was 0.255 with 95% CI [0.077, 0.846] and p=0.026 (table 1, figure 5). The Δex 25-27 splice variants introduces a frame shift in exon 28 resulting in a deletion of the C-terminal tail (exons 25-28) of EGFR. We therefore included C-terminal truncating mutations (SNVs leading to frameshifts and premature termination codons and fusion genes at the ‘3 end of the gene) in this analysis and found that the association remained significant.
(HR 0.175, 95% CI [0.054, 0.574], p = 0.004 for the combination of depatux-m + TMZ). The association also remained significant when other 3’ mutations were included in the analysis: 3’ end missense mutations (n=10) center around two hotspots at amino acids 993-1014 and 1065-1070 and it is possible that they affect a functional domain similar to the domain lost in the truncating mutations (table 1, figure 5). Exon deletions overlapping the hotspots Δex 25-26 or Δex 27, identified in 14 and 5 samples respectively, may affect a similar domain. When all C-terminal mutations were combined (Δex 25-28, Δex 25-26, Δex 27, protein truncation SNVs and/or other 3’SNVs fusion genes, n=28 samples) the HR for depatux-m + TMZ was 0.309 [0.130, 0.735], p = 0.008. Depatux-m monotherapy was not significantly associated with survival (HR 0.514 [0.229, 1.155], p = 0.107, table 1, figure 5). We acknowledge that care should be taken when conducting such post-hoc and combinatorial analyses, therefore, the analysis of each variant type is listed separately (table 1).

Expression of EGFRvIII is common in glioblastomas and, since depatux-m also has affinity for this deletion variant, we were particularly interested in association with survival. In contrast to what might be expected, the absence of EGFRvIII expression showed a trend towards association with survival: the HR was 0.582, 95% CI [0.311, 1.088], p = 0.090 in the combination arm and 1.004, 95% CI [0.570, 1.771], p = 0.988 in the depatux-m monotherapy arm both compared to the CCNU or TMZ control arm (table 1, figure 3, all treatment arms shown in supplementary figure 2). Multivariable analysis including MGMT promoter methylation status and age confirmed the trend towards association of depatux-m + TMZ with survival in samples without EGFRvIII expression-SNVs (HR 0.57, 95% CI [0.31, 1.03], p = 0.064, supplementary table 1).

Because MGMT status is predictive for response to TMZ chemotherapy, we stratified the molecular markers associated with survival (EGFR-SNVs and absent EGFRvIII expression) by this factor. Although sample size is relatively small, both depatux-m + TMZ and MGMT promoter methylation status were
associated with improved outcome and both associations remained significant in a multivariable analysis containing both factors (supplementary figure 3).

*Extracellular missense mutations result in a receptor with increased ligand sensitivity*

We next aimed to determine why SNVs in EGFR are associated with response to depatux-m + TMZ. First, the presence of SNVs and absence of EGFRvIII expression identify a similar population: the majority of samples expressing EGFRvIII do not express additional EGFR mutation variants (18/96) and the majority of samples harboring SNVs do not express EGFRvIII (18/57), $P=0.004$ (Chi square test, figure 3c). The level of EGFRvIII expression is also lower in samples expressing SNVs: The spliced-in fraction (the number of mutant reads as fraction of the total EGFR reads) was $0.12 \pm 0.22$ vs $0.29 \pm 0.36$, $P<0.001$. Conversely, the level of EGFR SNVs was lower in samples expressing EGFRvIII compared to those that do not express EGFRvIII (spliced-in fraction, calculated only using reads covering the affected base, was $0.11 \pm 0.22$ vs $0.35 \pm 0.38$, $P<0.001$). The difference in expression is even larger when only focusing on hotspot mutations (i.e. any mutation occurring in at least four samples) where the spliced in fraction of EGFRvIII was $0.09 \pm 0.18$ vs $0.45 \pm 0.33$ in mutation positive vs negative tumors ($p<0.001$). The inverse correlation between expression of EGFRvIII and SNVs was not specific to samples included in this study; glioblastoma samples included in the randomized phase II BELOB trial and in TCGA samples show a near identical and statistically significant inverse correlation supplementary figure 3. This inverse correlation suggests a divergent evolution where, after the initial amplification of EGFR, glioblastomas develop either EGFRvIII or other EGFR mutations.

To understand why SNVs in EGFR are associated with survival in the depatux-m + TMZ arm, we performed functional analysis on various EGFR-mutation constructs. The majority of hotspot mutations in glioblastomas are localized in the extracellular, ligand binding domain of EGFR, and we hypothesized they may affect ligand-induced activation. We therefore stimulated various mutation
constructs with two different EGFR ligands, one high affinity ligand (EGF) and one low affinity ligand (Amphiregulin, AREG). Stimulation with 200 ng/ml EGF resulted in a strong increase EGFR-phosphorylation in all constructs (except for EGFRvIII; this constitutively active variant is ligand independent \(^{22,23}\)) (figure 4, 6). In contrast, the low affinity EGFR ligand amphiregulin (AREG, 200 ng/ml) only marginally activated the control constructs EGFR-wt and EGFR\(^{V834L}\) and did not activate EGFRvIII. Interestingly, AREG stimulation had a strong effect on all constructs harboring extracellular missense mutations found in glioblastomas (EGFR\(^{R108K}\), EGFR\(^{G598V}\), EGFR\(^{A289V}\)). AREG activated these EGFR-mutation constructs at levels 40%-80% of that observed by EGF stimulation, a 3-8 fold increase compared to EGFRwt constructs (p<0.02 for any extracellular mutation construct vs EGFRwt and p<0.05 for any of the extracellular mutation constructs vs EGFR\(^{V834L}\), figure 4). Mutations commonly found in lung cancer (EGFR\(^{L858R}\) and EGFR\(^{A746-E750\text{del}}\)) responded to EGF and AREG similar to the control constructs.

To determine whether extracellular domain mutations were also more sensitive to other EGFR ligands, we tested all seven known ligands for EGFR activation. For each ligand, we performed a dose response analysis ranging from 200 ng/ml (maximal stimulation) to 0.8 ng/ml. In the control constructs, all high affinity EGFR ligands (EGF, TGF\(\alpha\), HB-EGF and BTC) resulted in a strong activation and all low affinity ligands AREG, EREG and EPGN resulted in a markedly weaker activation (figure 6). In contrast however, all extracellular missense mutations, EGFR\(^{A298V}\), EGFR\(^{G598V}\) and EGFR\(^{R108K}\), showed strong activation towards all EGFR ligands, including the low affinity ligands AREG, EPGN, and EREG (figure 6). EGFRvIII did not respond to any of the ligands. These experiments therefore show that EGFR containing extracellular missense mutations render the receptor more sensitive to stimulation, especially by the weak activators AREG, EREG and EPGN.

The hypersensitivity of extracellular domain mutations may explain the increased responsiveness to depatux-m: receptors are more easily activated and, as activation leads to receptor internalization, increased internalization with the antibody/drug conjugate. Hypersensitivity likely also leads to an increased exposure of the epitope for the antibody (i.e. the activated conformation of EGFR) \(^{24}\).
Our molecular imaging analysis also showed that EGFRvIII mainly has an intracellular localization (supplementary figure 4). This is in contrast to control constructs which are mainly localized to the membrane. Other activating mutations such as EGFR<sup>L858R</sup> also showed a certain degree of increased intracellular localization, but only EGFRvIII showed such prominent intracellular localization. The increased responsiveness in samples without EGFRvIII expression therefore can be explained by the near absence of EGFRvIII on the extracellular membrane: this may prevent effective binding to depatux-m.

Functional analysis indeed confirmed the direct correlation between receptor internalization and EGFR antibody internalization: EGFR receptor internalization still occurred in the presence of either ABT806 or cetuximab, regardless of ligand or mutation present, whereas this internalization could be completely inhibited by erlotinib or lapatinib (respectively type I and II tyrosine kinase inhibitors). The internalization was accompanied by uptake of EGFR antibodies as demonstrated by staining cells only with secondary antibodies directed at the FC fragment (supplementary figure 6).

**Additional genetic events associated with survival in the depatux-m + TMZ arm**

We also performed correlative analysis to screen for other events associated with patient survival in the depatux-m + TMZ arm. We focussed on genetic events (SNVs and CNVs) present in at least nine samples and selected those showing a trend (p<0.10) by Cox regression analysis. Of the 149 genes examined, inactivating PTEN mutations were associated with outcome to depatux-m + TMZ; the HR was 0.499 with 95% CI [0.241, 1.034], p=0.061 (table 1, supplementary figure 5). For this analysis, we combined samples with homozygous deletion and SNVs (individual mutation types showed a similar trend, table 1). Combining homozygous deletion with SNVs was warranted as many of the SNVs in PTEN led to premature stop codons and most of the missense mutations are listed in the COSMIC database with high pathogenic prediction scores (FATHMM) (supplementary table 2).

A second gene associated with survival was ARID1A, a tumor suppressor gene that is mutated in various cancer types including ovarian, endometrial and uterine cancer. Mutations in this gene are
often heterozygous which suggests that inactivation of one allele is sufficient to relieve the tumor suppressive effect of the protein \(^\text{27}\). The identified SNVs in \textit{ARID1A} in our samples were also heterozygous. When combining samples with \textit{ARID1A} LOH (n=10) and missense mutations (n=12), the HR for depatux-m + TMZ was 0.27 (95% CI [0.074, 0.961], p= 0.04) (table 1, supplementary figure 6, analysis of LOH and SNVs individually are also listed). SNVs in dihydro-folate reductase (DHFR, a gene required for the de novo synthesis of purines) and RP11.770J1.4 (a long intragenic noncoding RNA) were also associated with response to the combination of depatux-m + TMZ (HR 0.587, p=0.050 and HR 0.322, p=0.057 respectively, supplementary figure 6).

Because inactivating alterations in PTEN and TP53 are associated with EGFR kinase inhibitor response in several cancer types (see e.g. \(^\text{28}\)), we performed a multivariate analysis including these genes and show that depatux-m + TMZ remained a factor associated with survival (supplementary table 3).

\textit{Gene expression analysis}

Whole transcriptomic analysis identified genes associated with survival in each of the three treatment arms (supplementary tables 4-6). For each of these gene-lists, several genes were co-expressed which is suggestive for higher order interactions (supplementary figure 7) Gene-set enrichment analysis identified various pathways associated with survival including ‘cell cycle’, ‘cell activation’ and ‘meiotic cell cycle process’, and various pathways associated with immune response including ‘immune system development’ and ‘lymphocyte activation’. We did not find a correlation between the level of immune infiltration and survival as determined by Immunophenoscore analysis\(^\text{29}\). One gene, N-MYC downstream regulated gene 2 (\textit{NDRG2}), was specifically associated with survival in the depatux-m + TMZ arm and not in the other two arms of the study (supplementary figure 6) suggesting this gene is predictive for response to the combination treatment. Other studies have also shown similar correlation between expression and survival of \textit{NDRG2} in glioblastomas\(^\text{30}\).
The survival curves in both depatux-m treated arms shows a tail suggesting more long survivors (>365 days from randomization) when treated with the drug. Gene expression analysis between long and short survivors identified 15 differentially expressed genes, including CDK4 and 6 genes that neighbour it. We find approximately 2.2 times more CDK4-amplified samples in patients with survival < 365 days (p=0.004, see also 31). Expression of FOXF1 was significantly higher in short- compared to long survivors (p=0.010, supplementary figure 8).
Discussion

In this study, we have performed detailed molecular analyses on glioblastomas of patients treated within the INTELLANCE-2/EORTC_1410 randomized phase II clinical trial. Our results suggest that patients harbouring tumors with EGFR SNVs may derive more benefit from the combination of depatux-m + TMZ.

Three mechanisms can explain the survival benefit of these variants to depatux-m + TMZ. First, we show that extracellular domain mutations result in a receptor that is hypersensitive to activation by the various EGFR ligands. Since EGFR is internalized after receptor activation\(^\text{32}\), the hypersensitivity likely increases internalization and so increase uptake of the antibody-drug conjugate. Second, hypersensitive mutations increase transformation towards the active conformation of the protein. EGFR can switch between inactive (closed) and active (domain II exposed) conformation; the presence of ligand locks the protein in the active conformation\(^\text{20,33}\). Hypersensitive mutations may shift the equilibrium towards the active conformation of the protein. Indeed, such mutations have been demonstrated to increase exposure of the epitope for the ABT806 antibody, and so result in increased binding of depatux-m\(^\text{24}\). Thirdly, we show that EGFRvIII expression is inversely correlated with the presence of SNVs in EGFR. Since EGFRvIII mainly has an intracellular localization (see also \(^\text{34}\)), its near absence on the extracellular membrane may prevent binding to depatux-m, samples without EGFRvIII expression (i.e. predominantly those with EGFR SNVs) are more likely to respond to depatux-m. Other mechanisms however, may also determine sensitivity/resistance\(^\text{35}\).

Our results are in line with two recent publications both using mice engrafted with cell lines specifically overexpressing either EGFR\(^\text{A289V}\) or EGFR\(^\text{G598V}\) ECD mutations. Both studies showed significant survival benefit from treatment with ABT806 (i.e. the antibody used in depatux-m)\(^\text{24,36}\). Since our study also suggested benefit when such mutations are present, and given the mechanistic insight of its possible mode of action, further investigation into the efficacy of depatux-m in glioblastoma patients with ECD mutations is warranted.
We also show that intracellular EGFR truncating mutations and splice variants are associated with response to depatux-m + TMZ. Other studies have shown that such truncating mutations result in altered receptor internalization\textsuperscript{37,38} and this altered internalization therefore may be linked to treatment response and survival benefit. Similarly, bi-allelic inactivation of PTEN also was associated with response to depatux-m + TMZ. Since poly-phosphoinositides are key regulators of membrane trafficking, they also may contribute to altered receptor endocysis. PtdIns(4,5)P\textsubscript{2} for example, is required in the progression of early endocytosis\textsuperscript{39} and PtdIns(4,5)P\textsubscript{2} is produced from PTEN substrate PtdIns(3,4,5)P\textsubscript{3}.

One important caveat of this study is that the correlative analysis are post-hoc, and therefore these observations require confirmation in an independent dataset: our analysis may have incorrectly identified markers associated with survival in the depatux-m + TMZ arm due to multiple testing. Such dataset may be available in the INTELLANCE-1 trial that examined the effect of depatux-m in combination with chemoradiation compared to chemoradiation only in newly diagnosed glioblastoma patients (clinicaltrials.gov identifier NCT02573324). Of note, this trial did not meet its primary endpoint, though trial results have not been published to date. The difference between the two trials may lie in the fact that the patients treated within INTELLANCE-1 received surgery prior to treatment. The remaining tumor cells in INTELLANCE-1 therefore were likely in areas with an intact blood-brain barrier, which may have reduced accessibility to depatux-m. In addition, most of the samples analysed in current study were derived from primary tumors (in \textasciitilde80\% of cases) as surgery at recurrence is seldom performed. Various changes can occur during tumor evolution and some specifically affect EGFR variants\textsuperscript{40-42}. \textit{EGFRvIII} expression for example, is often lost at tumor recurrence\textsuperscript{43-45}; \textasciitilde1/3 of EGFR-mutations are also lost at tumor recurrence \textsuperscript{46}. Nevertheless, the copy number changes, gene amplifications/deletions and mutations are similar to observed in other (EGFR-amplified) glioblastoma datasets and therefore patients included in the INTELLANCE-2/EORTC_1410 trial did not select for a specific and molecularly defined tumor type\textsuperscript{1}. Another limitation of our study is the that response is
extrapolated from survival data. Correlation of response as seen on MRI with molecular features may identify different genetic markers.

Our data also provides a model for tumor evolution with respect to EGFR-dependency. GBMs require EGFR signaling for growth and EGFR amplification is the first step in GBMs to meet this requirement. After this initial amplification, the tumor evolves to facilitate the need for EGFR signaling by gaining additional and activation mutations. Our data show that there are at least two different modes of evolution in glioblastomas: by becoming independent of ligand (EGFRvIII) or by becoming hypersensitive to ligand (extracellular hotspot mutations). Since both EGFRvIII and extracellular domain mutations have tumorigenic properties, only one of these mutations is required to facilitate the need for EGFR-signaling. This explains why, in all the glioblastoma datasets examined, EGFRvIII expression is inversely correlated with presence of EGFR-missense mutations (figure 3 and supplementary figure 2).

In addition to finding associations of specific EGFR variants associated with response to depatux-m + TMZ, we also found rare mutations that, in pulmonary adenocarcinoma patients, respond to EGFR-TKIs. Several lines of evidence suggest that responses to EGFR-TKIs are dependent on the type of mutation and not on the type of tumor. For example, TKI-sensitive mutations in pulmonary adenocarcinomas are also sensitive to these TKIs in other tumor types. Such responses have also been documented for the mutations identified in this study. These TKI-sensitive mutations have been identified in other glioblastoma datasets, and although present in only a small minority (~1-2%) of EGFR-amplified GBMs, EGFR-TKIs may prove an interesting treatment option for patients harbouring such tumors. However, one complicating factor is that EGFR mutations in glioblastomas almost invariably are subclonal. In addition, they often show high intratumoral (and temporal)
heterogeneity\textsuperscript{46}. It is therefore possible that EGFR-TKIs may only be effective in tumors with a high variant allele fraction of the TKI-sensitive mutation.
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Figure legends

Figure 1: Genomic landscape of samples included in the INTELLANCE-2/EORTC_1410 trial. Shown are waterfall plots of chromosomal changes (A), gains and losses of individual genes (B) and SNVs within individual genes (C). The copy number changes, gene amplifications/deletions and mutations are similar to observed in other (EGFR-amplified) glioblastoma datasets. Patients included in this study therefore were not selected for a specific molecularly subtype. LOH: loss of heterozygosity, HD: homozygous deletion.

Figure 2: Genetic changes within the EGFR gene of samples from patients included in the INTELLANCE-2/EORTC_1410 trial. A) lolliplot of SNVs identified showing characteristic hotspot mutations in the extracellular domain. B) waterfall plots of genetic changes subdivided in copy number gains, splice variant expression, and mutations. The waterfall plot is colour coded to represent the level of copy number gain (in the CNV plot) or the percentage of mutant alleles (percentage spliced in).

Figure 3: Genetic changes within the EGFR gene that were associated with prolonged survival with depatux-m + TMZ. A) presence of SNVs; B) absence of EGFRvIII expression. Hazard rates of these changes are listed in table 1. Both genetic changes are correlated as samples containing SNVs often do not express EGFRvIII and vice versa (C).

Figure 4: A) A289x hotspot mutations are associated with response to depatux-m + TMZ. B) example of microscopic images of EGFR-wt and EGFRR108K stimulated with either EGF or AREG. Green: EGFR, red: phospho-EGFR, Blue: Hoechst. AREG resulted in increased receptor endocytosis and phospho-EGFR signal in EGFRR108K compared to EGFR-wt. C) Example of analysis depicting the level of phospho-EGFR (y-axis) against total EGFR (x-axis) within individual EGFR positive vesicles. Each dot represents one EGFR-positive submenbranous vesicle. D) averages of experiments plotted in C. E) Similar to B) the level of phospho-EGFR (y-axis) is plotted against total EGFR (x-axis) within individual EGFR positive vesicles. As can be seen, apart from EGFRvIII, all constructs responded, dose dependently, to the high
affinity ligands EGF, HB-EGF, TGFA and BTC by an increase in EGFR phosphorylation. Low affinity ligands AREG, EREG and EPGN were not able to stimulate EGFR-wt constructs, but resulted in a strong activation of extracellular domain mutations EGFR<sup>R108K</sup>, EGFR<sup>A289V</sup> and EGFR<sup>G598V</sup>. The absence of responses in EGFRvIII expressing cells is in line with the notion that this mutation is independent of ligand. Images were taken at 40x magnification.

**Figure 5:** C-terminal truncating mutations are associated with response to depatux-m + TMZ. Deletion of exons 25-27 result in a frame shift of the protein. Such changes are associated with response to depatux-m + TMZ (top right). Nonsense and frameshift mutations show a similar trend (middle panels, left lolliplot of individual mutations, right survival analysis), as do the C-terminal SNVs (bottom panels, left lolliplot of individual mutations, right survival analysis). Hazard rates of individual changes are listed in table 1.
### Supplementary table 1 multivariable analysis

|                | HR   | 95% CI    | p     |
|----------------|------|-----------|-------|
| **EGFR SNV present** |      |           |       |
| depatux-m      | 0.718| 0.430     | 1.199 | 0.206 |
| TMZ+depatux-m  | 0.448| 0.263     | 0.760 | 0.003 |
| MGMT Methylated| 0.447| 0.285     | 0.702 | 0.000 |
| Age >40, <60   | 1.128| 0.316     | 2.488 | 0.819 |
| Age >60        | 1.434| 0.508     | 4.053 | 0.496 |
| **EGFRvIII absent** |      |           |       |
| depatux-m      | 1.096| 0.601     | 1.999 | 0.764 |
| TMZ+depatux-m  | 0.566| 0.310     | 1.033 | 0.064 |
| MGMT Methylated| 0.519| 0.315     | 0.853 | 0.010 |
| Age >40, <60   | 1.030| 0.308     | 3.443 | 0.962 |
| Age >60        | 1.62568| 0.4952 | 5.337 | 0.423 |
## Supplementary table 2: PTEN mutations in intelligence 2 samples.

| PATID | sex | treatment arm | censor | OS (months) | COSMIC_ID          | variantFreq | consequence | Mutation               |
|-------|-----|---------------|--------|-------------|--------------------|--------------|-------------|------------------------|
| 68    | F   | dep-m         | 1      | 19.0        | COSM1675407        | 0.721        | missense    | p.Tyr46Cys             |
| 88    | M   | TMZ+dep-m     | 1      | 3.1         | COSM5027           | 0.886        | missense    | p.Asp162Glu            |
| 107   | M   | TMZ+dep-m     | 1      | 3.7         | COSM28917          | 0.534        | missense    | p.Gly129Glu            |
| 124   | M   | TMZ+dep-m     | 1      | 1.1         |                    | 0.738        | frameshift  | p.Ile101SerfsTer12    |
| 149   | F   | TMZ|CCNU       | 1      | 4.2         | COSM44896          | 0.736        | frameshift  | p.Thr319LysfsTer24    |
| 178   | F   | dep-m         | 1      | 3.8         | COSM5109           | 0.677        | missense    | p.Ile101Thr            |
| 188   | M   | dep-m         | 0      | 18.7        |                    | 0.368        | missense    | p.Arg159Gly            |
| 191   | F   | TMZ|CCNU       | 1      | 19.1        | COSM23643          | 0.468        | frameshift  | p.Glu106Ter            |
| 191   | F   | TMZ|CCNU       | 1      | 19.1        | COSM30611          | 0.381        | missense    | p.Asp107Tyr            |
| 191   | F   | TMZ|CCNU       | 1      | 19.1        | COSM23643          | 0.422        | stop_gained | p.CysGlu105TerGln     |
| 267   | M   | dep-m         | 1      | 3.4         | COSM13459          | 0.722        | frameshift  | p.Ser113ArgfsTer2     |
| 295   | M   | TMZ|CCNU       | 1      | 2.9         | COSM249839         | 0.762        | missense    | p.Tyr68Asn             |
| 299   | M   | TMZ+dep-m     | 1      | 9.4         | COSM1173628        | 0.375        | stop_gained | p.Arg233Ter            |
| 321   | M   | TMZ+dep-m     | 1      | 24.1        |                    | 0.503        | missense    | p.Gly209Glu            |
| 322   | F   | TMZ+dep-m     | 1      | 6.0         | COSM23566          | 0.843        | missense    | p.Asp92Tyr             |
| 323   | M   | TMZ|CCNU       | 1      | 8.9         | COSM4894833        | 0.609        | frameshift  | p.Glu43LysfsTer11      |
| 330   | M   | TMZ|CCNU       | 1      | 5.2         | COSM4995           | 0.602        | missense    | p.Cys136Arg            |
| 340   | M   | dep-m         | 1      | 14.3        | COSM1627662        | 0.222        | frameshift  | p.Cys296Ter            |
| 361   | M   | dep-m         | 1      | 7.1         | COSM5347060        | 0.654        | missense    | p.Phe195Ser            |
| 375   | M   | dep-m         | 0      | 18.2        | COSM5033           | 0.816        | missense    | p.Arg130Gln            |
| 375   | M   | dep-m         | 0      | 18.2        | COSM5033           | 0.288        | frameshift  | p.Arg130GlnfsTer4      |
| 391   | F   | TMZ|CCNU       | 1      | 8.0         | COSM249825         | 0.728        | stop_gained | p.Gly127Ter            |
| 396   | F   | dep-m         | 1      | 8.4         | COSM5347090        | 0.226        | missense    | p.His259Pro            |
| 418   | M   | TMZ+dep-m     | 1      | 6.8         | COSM5151           | 0.159        | stop_gained | p.Arg335Ter            |
| 435   | M   | TMZ|CCNU       | 1      | 13.9        | COSM5160           | 0.704        | stop_gained | p.Gln261Ter            |
| 447   | M   | TMZ|CCNU       | 1      | 7.4         | COSM5157           | 0.759        | missense    | p.Trp111Ser            |
| 454   | M   | TMZ|CCNU       | 1      | 8.8         | COSM921142         | 0.629        | frameshift  | p.Ile300MetsfsTer7     |
| 457   | M   | TMZ+dep-m     | 0      | 10.6        | COSM1666994        | 0.747        | missense    | p.Met35Val             |
| 490   | M   | dep-m         | 1      | 3.0         | COSM1173628        | 0.587        | stop_gained | p.Arg233Ter            |
| 512   | M   | TMZ+dep-m     | 1      | 4.5         | COSM4898           | 0.630        | frameshift  | p.Thr319Ter            |
| 538   | F   | TMZ|CCNU       | 1      | 6.5         | COSM1666994        | 0.467        | missense    | p.Met35Val             |
| 555   | F   | dep-m         | 1      | 5.9         | COSM4936           | 0.652        | frameshift  | p.Phe347SerfsTer5      |
| 576   | M   | dep-m         | 1      | 1.9         | COSM921127         | 0.399        | frameshift  | p.Pro248ThrfsTer5      |
| 594   | M   | TMZ+dep-m     | 1      | 18.3        | COSM4936           | 0.623        | frameshift  | p.Phe347TyrfsTer13     |
| 631   | M   | TMZ|CCNU       | 1      | 9.6         | COSM428079         | 0.273        | missense    | p.Arg47Lys             |
| 636   | M   | dep-m         | 1      | 17.8        | COSM13578          | 0.445        | missense    | p.Cys124Tyr            |
| 649   | M   | dep-m         | 1      | 5.5         | COSM1744939        | 0.891        | frameshift  | p.Asn329LysfsTer14     |
| 657   | F   | dep-m         | 1      | 1.2         | COSM4963           | 0.569        | missense    | p.Tyr76Asn             |
| 695   | M   | TMZ+dep-m     | 0      | 20.6        | COSM1173647        | 0.232        | missense    | p.Pro246Leu            |
| 696   | M   | TMZ+dep-m     | 0      | 20.3        | COSM5295           | 0.534        | stop_gained | p.Ser229Ter            |
| 730   | F   | dep-m         | 0      | 21.0        | COSM23654          | 0.631        | frameshift  | p.Glu235LysfsTer21     |
| 768   | M   | dep-m         | 0      | 19.5        | COSM5090           | 0.620        | missense    | p.Pro96Leu             |
| 786   | M   | dep-m         | 1      | 7.8         | COSM5151           | 0.455        | stop_gained | p.Arg335Ter            |
|    |   |           |   |          |       |       |       |              |              |              |
|----|---|------------|---|-----------|-------|-------|-------|----------------|----------------|----------------|
| 791| F | TMZ+dep-m  | 0 | 19.2      | COSM5033 | 0.533 | missense | p.Arg130Gln    |                |                |
| 816| F | TMZ+dep-m  | 1 | 5.4       | COSM1173628 | 0.731 | stop_gained | p.Arg233Ter    |                |                |
| 867| M | TMZ+dep-m  | 1 | 4.1       | COSM5163 | 0.740 | stop_gained | p.Gln87Ter     |                |                |
| 869| M | TMZ+dep-m  | 0 | 19.3      |         | 0.609 | frameshift | p.Asn311Ter    |                |                |
| 892| M | TMZ|CCNU      | 1 | 9.4       | COSM28884 | 0.399 | missense | p.Ala126Gly    |                |                |
| 919| F | TMZ+dep-m  | 1 | 8.9       | COSM5082 | 0.381 | missense | p.Lys125Gln    |                |                |
| 962| M | dep-m      | 1 | 0.2       |         | 0.699 | missense | p.Gly209Glu    |                |                |
| 974| M | TMZ|CCNU      | 1 | 10.2      | COSM4408405 | 0.479 | missense | p.Ile33Thr     |                |                |
| 976| M | TMZ|CCNU      | 1 | 7.0       | COSM35849 | 0.729 | frameshift | p.Glu242Ter    |                |                |
| 999| M | TMZ+dep-m  | 1 | 2.2       | COSM5313 | 0.546 | stop_gained | p.Ser59Ter     |                |                |
|1017| M | TMZ+dep-m  | 1 | 11.0      | COSM5103 | 0.701 | missense | p.Gly156Arg    |                |                |
|1032| M | TMZ+dep-m  | 0 | 18.4      | COSM5039 | 0.612 | missense | p.Arg173His    |                |                |
|1057| M | TMZ+dep-m  | 0 | 17.9      | COSM3736942 | 0.192 | missense | p.Leu247Ser    |                |                |
|1062| M | TMZ+dep-m  | 1 | 6.1       | COSM27635 | 0.418 | missense | p.Phe341Cys    |                |                |
|1065| F | dep-m      | 1 | 4.2       | COSM5291 | 0.734 | stop_gained | p.Tyr225Ter    |                |                |
|1077| M | dep-m      | 1 | 10.5      | COSM1744939 | 0.696 | frameshift | p.Asn329LysfsTer14 |                |                |
|1083| M | dep-m      | 1 | 9.0       | COSM5105 | 0.536 | missense | p.Pro95Leu     |                |                |
|1096| M | TMZ+dep-m  | 1 | 17.3      | COSM428084 | 0.358 | stop_gained | p.Tyr88Ter     |                |                |
|1099| F | TMZ+dep-m  | 1 | 2.0       | COSM5123 | 0.828 | missense | p.Gly132Asp    |                |                |
|1110| M | dep-m      | 1 | 13.0      | COSM308347 | 0.363 | stop_gained | p.Arg130Ter    |                |                |
|1120| M | TMZ+dep-m  | 1 | 15.4      | COSM921068 | 0.463 | missense | p.Asp52Val     |                |                |
|1128| F | TMZ+dep-m  | 1 | 6.3       | COSM1173628 | 0.902 | stop_gained | p.Arg233Ter    |                |                |
Supplementary table 3: Multivariable analysis on PTEN and TP53

|                          | HR  | 95% CI  | p     |
|--------------------------|-----|---------|-------|
| depatux-m                | 0.867 | 0.601 | 1.252 | 0.447 |
| TMZ+depatux-m            | 0.603 | 0.416 | 0.875 | 0.008 |
| MGMT Methylated          | 0.550 | 0.402 | 0.752 | 0.000 |
| PTEN LOH                 | 0.874 | 0.422 | 1.809 | 0.717 |
| PTEN wt                  | 0.749 | 0.289 | 1.939 | 0.552 |

|                          | HR  | 95% CI  | p     |
|--------------------------|-----|---------|-------|
| depatux-m                | 0.872 | 0.600 | 1.266 | 0.470 |
| TMZ+depatux-m            | 0.615 | 0.419 | 0.903 | 0.013 |
| MGMT Methylated          | 0.534 | 0.390 | 0.732 | 0.000 |
| TP53 mut                 | 1.561 | 1.038 | 2.346 | 0.032 |
### Supplementary table 4: Genes associated with overall survival in the CCNU|TMZ arm

| gene.id             | Beta     | Z        | P        | Wald     | HR       |
|---------------------|----------|----------|----------|----------|----------|
| AC078906.1|chr8:72M | 1.4571   | 4.0197   | 0.0001   | 0.0001   | 4.2933   |
| DLGAP1|chr18:4M   | 0.4927   | 4.0999   | 0        | 0        | 1.6368   |
| TIAM2|chr6:155M  | 0.8737   | 4.0329   | 0.0001   | 0.0001   | 2.3957   |
| MIR3691|chr6:5M    | 1.8868   | 3.8936   | 0.0001   | 0.0001   | 6.5985   |
| AC026358.1|chr12:124M| 0.9745   | 3.8199   | 0.0001   | 0.0001   | 2.6498   |
| ZNF518B|chr4:10M   | -0.447   | -3.6262  | 0.0003   | 0.0003   | 0.6395   |
| CACNG4|chr17:67M  | 0.3723   | 3.5885   | 0.0003   | 0.0003   | 1.4511   |
| THAP12P2|chr3:86M   | 0.6191   | 3.573    | 0.0004   | 0.0004   | 1.8573   |
| KLF11|chr2:10M   | -0.7748  | -3.5452  | 0.0004   | 0.0004   | 0.4608   |
| AC092111.1|chr12:8M  | 0.7364   | 3.4893   | 0.0005   | 0.0005   | 2.0884   |
| AC078842.1|chr7:137M | 0.8516   | 3.5559   | 0.0004   | 0.0004   | 2.3433   |
| MPPED2|chr11:30M  | 0.4112   | 3.4334   | 0.0006   | 0.0006   | 1.5087   |
| LINGO1-AS1|chr15:78M | 0.8874   | 3.4435   | 0.0006   | 0.0006   | 2.4288   |
| PLPPR5|chr1:99M   | 0.4524   | 3.3994   | 0.0007   | 0.0007   | 1.572    |
| CHRNA6|chr8:43M   | -0.9506  | -3.3905  | 0.0007   | 0.0007   | 0.3865   |
| AC021212.1|chr5:99M  | 1.0861   | 3.4171   | 0.0006   | 0.0006   | 2.9627   |
| KCNMB1|chr5:170M  | -0.7434  | -3.4392  | 0.0006   | 0.0006   | 0.4755   |
| SR5F12|chr6:89M   | 0.6848   | 3.4171   | 0.0006   | 0.0006   | 1.9833   |
| TNFSF10|chr3:173M  | -0.6256  | -3.4294  | 0.0006   | 0.0006   | 0.5349   |
| TRIM54|chr2:27M   | 0.841    | 3.4064   | 0.0007   | 0.0007   | 2.3188   |
| ZNF485|chr10:44M  | -1.1614  | -3.3717  | 0.0007   | 0.0007   | 0.3131   |
| HPX|chr11:6M   | 0.8625   | 3.3836   | 0.0007   | 0.0007   | 2.3691   |
| P2RY4|chrX:70M   | 1.0243   | 3.3601   | 0.0008   | 0.0008   | 2.785    |
| AFDN|chr6:168M  | 0.9076   | 3.3759   | 0.0007   | 0.0007   | 2.4785   |
| ST8SIA5|chr18:47M  | 0.2713   | 3.3194   | 0.0009   | 0.0009   | 1.3116   |
| AC027801.1|chr15:50M | 0.5133   | 3.3209   | 0.0009   | 0.0009   | 1.6707   |
| AC124947.1|chr12:93M| -1.8773  | -3.2929  | 0.001    | 0.001    | 0.153    |
| GREB1|chr2:12M   | 0.49     | 3.3113   | 0.0009   | 0.0009   | 1.6323   |
| DISP2|chr15:40M  | 0.4255   | 3.3284   | 0.0009   | 0.0009   | 1.5303   |
| AF121898.1|chr5:88M  | 1.3601   | 3.2639   | 0.0011   | 0.0011   | 3.8966   |
| GAD1|chr2:171M  | 0.25     | 3.2604   | 0.0011   | 0.0011   | 1.284    |
| OACYP1|chr18:59M  | 0.6397   | 3.2863   | 0.001    | 0.001    | 1.8959   |
| ADAMTS7P4|chr15:85M | 0.6119   | 3.3071   | 0.0009   | 0.0009   | 1.8439   |
| BMS1P2|chr10:48M  | 0.9866   | 3.3293   | 0.0009   | 0.0009   | 2.6821   |
| LINC00475|chr9:92M | -0.4167  | -3.2173  | 0.0013   | 0.0013   | 0.6592   |
| ZNF662|chr3:43M   | 0.849    | 3.2276   | 0.0012   | 0.0012   | 2.3372   |
| AC108215.1|chr4:57M | 0.3885   | 3.2032   | 0.0014   | 0.0014   | 1.4747   |
| HEG1|chr3:125M  | -0.9015  | -3.2191  | 0.0013   | 0.0013   | 0.406    |
| NCAPD3|chr11:134M | 1.4635   | 3.2152   | 0.0013   | 0.0013   | 4.3212   |
| C2orf80|chr2:208M  | 0.4878   | 3.2069   | 0.0013   | 0.0013   | 1.6288   |
| ACSL5|chr10:112M | -0.6688  | -3.1932  | 0.0014   | 0.0014   | 0.5123   |
| Gene Symbol | Chromosome | Start Position | BMax | BMin | p-Value | FDR | Fold Change |
|-------------|------------|----------------|------|------|---------|-----|-------------|
| DRD2        | chr11:113M| 0.4139         | 3.1899 | 0.0014 | 0.0014 | 1.5127 |
| AP004608.1  | chr11:134M| 0.6228         | 3.2575 | 0.0011 | 0.0011 | 1.8642 |
| TTC24       | chr1:157M  | 0.6928         | 3.1743 | 0.0015 | 0.0015 | 1.9993 |
| HS6ST2      | chrX:133M  | 0.332          | 3.175  | 0.0015 | 0.0015 | 1.3937 |
| ABI3BP      | chr3:101M  | -0.274         | -3.1476 | 0.0016 | 0.0016 | 0.7603 |
| HAPLN1      | chr5:84M   | 0.3172         | 3.1293 | 0.0018 | 0.0018 | 1.3732 |
| TRMT9B      | chr8:13M   | 0.4713         | 3.1394 | 0.0017 | 0.0017 | 1.6021 |
| COL28A1     | chr7:7M    | 0.2533         | 3.119  | 0.0018 | 0.0018 | 1.2882 |
| ZNF433      | chr19:12M  | 0.8285         | 3.1524 | 0.0016 | 0.0016 | 2.2898 |
Supplementary table 5: Genes associated with survival in the deptux-m monotherapy arm

| gene.id         | Beta   | Z       | P         | Wald   | HR     |
|-----------------|--------|---------|-----------|--------|--------|
| TRIM22|chr11:6M | -1.0041 | -4.0092 | 0.0001 | 0.3664 |
| BANK1|chr4:102M | -0.9445 | -4.0117 | 0.0001 | 0.3889 |
| AC115522.1|chr19:44M | 1.6241 | 3.8821 | 0.0001 | 5.074  |
| VNN1|chr6:133M | -1.5262 | -4.0537 | 0.0001 | 0.2174 |
| XAF1|chr17:7M  | -0.4941 | -3.7976 | 0.0001 | 0.6101 |
| ZFHX3|chr16:73M | -1.5297 | -3.828  | 0.0001 | 0.2166 |
| PITHD1|chr12:113M | 1.4973 | 3.6849 | 0.0002 | 4.4695 |
| AL162431.1|chr1:181M | -1.4981 | -3.5314 | 0.0004 | 0.2235 |
| UBE3B|chr12:110M | -2.5423 | -3.517  | 0.0004 | 0.0787 |
| LBR|chr1:225M  | 0.9501  | 3.5107  | 0.0004 | 2.586  |
| KIAA1671|chr22:25M | -1.0162 | -3.497  | 0.0005 | 0.362  |
| EMC1|chr1:19M   | 1.4416  | 3.5236  | 0.0004 | 4.2275 |
| ATP5MGL|chr22:43M | -1.5804 | -3.4824 | 0.0005 | 0.2059 |
| FCO1|chr3:46M   | -1.1549 | -3.465  | 0.0005 | 0.3151 |
| TFR2|chr7:101M  | 0.531   | 3.4423  | 0.0006 | 1.7005 |
| Tmprss2|chr21:41M | -0.7576 | -3.3367 | 0.0008 | 0.4688 |
| Tmem221|chr19:17M | -0.6403 | -3.4369 | 0.0006 | 0.5272 |
| SSTR3|chr22:37M  | 0.8569  | 3.3997  | 0.0007 | 2.3557 |
| Mybphl|chr1:109M | 0.9547  | 3.473   | 0.0005 | 2.5978 |
| Ugt2|chr13:96M  | 0.8662  | 3.4205  | 0.0006 | 2.3778 |
| Rnf219|chr13:79M | 0.904   | 3.4486  | 0.0006 | 2.4695 |
| Zdhhc2|chr8:17M  | -0.7237 | -3.4311 | 0.0006 | 0.485  |
| Ac009831.1|chr18:32M | -1.426  | -3.4312 | 0.0006 | 0.2403 |
| Pyr1|chr17:82M  | 0.6483  | 3.3838  | 0.0007 | 1.9123 |
| Apobec3f|chr22:39M | -0.721  | -3.3344 | 0.0009 | 0.4863 |
| Gimap8|chr7:150M | -0.8466 | -3.3177 | 0.0009 | 0.4289 |
| Tnfsf10|chr3:173M | -0.6548 | -3.2858 | 0.001  | 0.5195 |
| Ip05|chr13:98M | 1.1989  | 3.279   | 0.001  | 3.3166 |
| Rf0091|chr9:46M  | 0.7265  | 3.2461  | 0.0012 | 2.0679 |
| Unc5a|chr5:177M | 0.4481  | 3.2576  | 0.0011 | 1.5653 |
| Tfrc|chr3:196M | 0.7306  | 3.2512  | 0.0011 | 2.0764 |
| Slc25a15|chr13:41M | 0.9712  | 3.2035  | 0.0014 | 2.6411 |
| Npas2|chr2:101M | -0.4377 | -3.2475 | 0.0012 | 0.6455 |
| Krt8|chr12:53M | -0.9224 | -3.2741 | 0.0011 | 0.3976 |
| KCNJ4|chr22:38M | 0.476   | 3.2011  | 0.0014 | 1.6096 |
| Flil|chr11:129M | -0.8218 | -3.2209 | 0.0013 | 0.4397 |
| Ac091826.2|chr5:88M | 1.027   | 3.2019  | 0.0014 | 2.7927 |
| Morc4|chrX:107M | -1.2272 | -3.2367 | 0.0012 | 0.2931 |
| Utrn|chr6:145M | -0.9107 | -3.2264 | 0.0013 | 0.4023 |
| Ccdc69|chr5:151M | -0.6505 | -3.181  | 0.0015 | 0.5218 |
| Gene       | Chr/MM     | Value 1  | Value 2  | p Value 1 | p Value 2 | FDR Value 1 | FDR Value 2 |
|------------|------------|----------|----------|-----------|-----------|-------------|-------------|
| EPHB6      | chr7:143M  | 0.4757   | 3.1677   | 0.0015    | 0.0015    | 1.6092      |             |
| ADA2       | chr22:17M  | -0.4813  | -3.1768  | 0.0015    | 0.0015    | 0.618       |             |
| SNORD91A   | chr17:2M   | 1.5316   | 3.1813   | 0.0015    | 0.0015    | 4.6255      |             |
| MAF        | chr16:80M  | -0.7646  | -3.1995  | 0.0014    | 0.0014    | 0.4655      |             |
| GBAP1      | chr1:155M  | 0.9165   | 3.1533   | 0.0016    | 0.0016    | 2.5005      |             |
| CASP10     | chr2:201M  | -0.9303  | -3.1604  | 0.0016    | 0.0016    | 0.3944      |             |
| LTA4H      | chr12:96M  | -1.1694  | -3.1557  | 0.0016    | 0.0016    | 0.3106      |             |
| RTBDN      | chr19:13M  | 1.3057   | 3.112    | 0.0019    | 0.0019    | 3.6901      |             |
| APOC1P1    | chr19:45M  | -1.1163  | -3.1376  | 0.0017    | 0.0017    | 0.3275      |             |
Supplementary table 6: Genes associated with survival in the deptux-m + TMZ combination arm

| gene.id       | Beta   | Z      | P       | Wald    | HR      |
|---------------|--------|--------|---------|---------|---------|
| YEATS2|chr3:184M | 1.3252  | 3.667   | 0.0002  | 0.0002  | 3.7628  |
| HIST1H4K|chr6:28M  | 0.9528  | 3.524   | 0.0004  | 0.0004  | 2.5929  |
| SLC2A4|chr14:92M | -0.3454 | -3.4523 | 0.0006  | 0.0006  | 0.708   |
| FOXN3|chr14:89M | -1.6641 | -3.3232 | 0.0009  | 0.0009  | 0.1894  |
| DNMBP|chr10:100M | 1.024   | 3.2998  | 0.001   | 0.001   | 2.7842  |
| AC005609.5|chr5:141M | -1.2471 | -3.2581 | 0.0011  | 0.0011  | 0.2873  |
| DGLUCY|chr14:91M | -1.3433 | -3.2956 | 0.001   | 0.001   | 0.261   |
| NDRG2|chr14:21M | -0.6052 | -3.2525 | 0.0011  | 0.0011  | 0.546   |
| HEATR4|chr14:74M | -0.6854 | -3.2447 | 0.0012  | 0.0012  | 0.5039  |
| DBF4P1|chr10:64M | 2.1913  | 3.2992  | 0.001   | 0.001   | 2.7842  |
| GHRL|chr3:10M  | -1.2013 | -3.2083 | 0.0013  | 0.0013  | 0.3008  |
| PEbp4|chr8:23M  | -1.2872 | -3.1392 | 0.0017  | 0.0017  | 0.276   |
| COL4A3|chr2:227M | -0.3814 | -3.1369 | 0.0017  | 0.0017  | 0.681   |
| VMAC|chr19:6M  | -1.0008 | -3.0733 | 0.0021  | 0.0021  | 0.3676  |
| GTF2H2B|chr5:70M  | 0.5182  | 3.0141  | 0.0026  | 0.0026  | 1.679   |
| LINC01285|chrX:119M | -0.6189 | -3.0685 | 0.0022  | 0.0022  | 0.5385  |
| DCHS2|chr1:7M   | 1.0314  | 3.0571  | 0.0022  | 0.0022  | 2.8051  |
| AL713852.1|chr1:185M | -0.6638 | -3.0049 | 0.0027  | 0.0027  | 0.5149  |
| LINC01198|chr13:46M | 0.6149  | 2.9892  | 0.0022  | 0.0022  | 1.8494  |
| MAD2L1BP|chr6:44M  | 1.464   | 3.0195  | 0.0025  | 0.0025  | 4.3231  |
| DCHS2|chr4:154M | -0.4987 | -2.9584 | 0.0031  | 0.0031  | 0.6073  |
| EML5|chr1:7M   | 1.0314  | 3.0571  | 0.0022  | 0.0022  | 2.8051  |
| XPOS|chr6:44M  | 1.6162  | 2.9655  | 0.003   | 0.003   | 5.034   |
| AL109615.1|chr6:44M  | 1.3522  | 2.9455  | 0.0032  | 0.0032  | 3.8658  |
| LINC00628|chr1:204M | 0.9164  | 2.8936  | 0.0038  | 0.0038  | 2.5003  |
| GUSBP9|chr5:71M  | 1.3134  | 2.9304  | 0.0034  | 0.0034  | 3.7187  |
| TMEM1311|chr4:154M | 0.6672  | 2.9144  | 0.0036  | 0.0036  | 1.9487  |
| AC006487.2|chr1:7M   | -1.1308 | -2.9185 | 0.0035  | 0.0035  | 0.3228  |
| FMO2|chr1:171M | -0.6225 | -2.9264 | 0.0034  | 0.0034  | 0.5366  |
| ZC3H11B|chr1:220M | 1.1361  | 2.9291  | 0.0034  | 0.0034  | 3.1145  |
| PEL1|chr14:56M | -0.7798 | -2.8948 | 0.0038  | 0.0038  | 0.4585  |
| FAM20B|chr1:179M | 1.2708  | 2.881   | 0.004   | 0.004   | 3.5639  |
| NAP1L2|chrX:73M  | -0.3979 | -2.8705 | 0.0041  | 0.0041  | 0.6717  |
| GSG1L|chr16:28M | -0.3134 | -2.8455 | 0.0044  | 0.0044  | 0.731   |
| HSD17B7P2|chr10:38M | 0.7211  | 2.8532  | 0.0043  | 0.0043  | 2.0568  |
| SMIM7|chr19:17M | -1.3593 | -2.8815 | 0.004   | 0.004   | 0.2568  |
| GGTA1P|chr9:121M | -0.4849 | -2.8945 | 0.0038  | 0.0038  | 0.6158  |
| TTY15|chrY:13M  | -0.3591 | -2.7624 | 0.0057  | 0.0057  | 0.6983  |
| KLHL31|chr6:54M  | -1.0894 | -2.8264 | 0.0047  | 0.0047  | 0.3364  |
| AC093752.3|chr4:119M | -1.0293 | -2.8155 | 0.0049  | 0.0049  | 0.3573  |
| Gene            | Chromosome | Start | Strand | Direction | Log2 Fold Change | p-value | q-value | FDR Value |
|-----------------|------------|-------|--------|-----------|-----------------|---------|---------|-----------|
| METTL7A         | chr12:51M |       |        |           | -0.4612         | 0.0051  | 0.0051  | 0.6305    |
| AC109361.1      | chr4:106M | -1.3182 |       |           | -2.8015         | 0.0047  | 0.0047  | 0.2676    |
| CABLES2         | chr20:62M | 0.8582 |       |           | 2.791           | 0.0053  | 0.0053  | 2.359     |
| TPGS2           | chr18:37M | 1.0585 |       |           | 2.759           | 0.0058  | 0.0058  | 2.8821    |
| PLSCR2          | chr3:146M | -0.8467 |       |           | -2.7271         | 0.0064  | 0.0064  | 0.4288    |
| ACO51619.3      | chr15:45M | -0.5114 |       |           | -2.7462         | 0.006   | 0.006   | 0.5997    |
| AP0000793.1     | chr11:83M | -0.8667 |       |           | -2.7259         | 0.0064  | 0.0064  | 0.4203    |
| DVL3            | chr3:184M | 1.2316 |       |           | 2.8266          | 0.0047  | 0.0047  | 3.4267    |
Supplementary figure 1: Lolliplot of hotspot mutations in TP53.
Supplementary figure 2: The absence EGFRvIII mutations are associated with response to depatux-m + TMZ. Listed are data from all treatment arms.
Supplementary figure 3: Markers associated with survival stratified by treatment arm and MGMT promoter methylation status. Because MGMT status is predictive for response to TMZ chemotherapy, the molecular markers associated with survival (EGFR-SNVs and absent EGFRvIII expression) were stratified for this factor. Although sample size is small, MGMT promoter methylation was associated with improved outcome in the various treatment arms. A) presence of EGFR SNVs stratified by MGMT promoter methylation status. B) absence of EGFRvIII expression stratified by MGMT promoter methylation status. Tables are included listing the multivariable analysis including treatment and MGMT status (top tables) and the median survival per group (bottom tables). Multivariable analysis confirms that both the combination treatment and MGMT promoter methylation are associated with improved survival.
Supplementary figure 4: Glioblastoma samples containing SNVs often do not express \textit{EGFRvIII} and vice versa in samples of the BELOB trial (top panel) and in TCGA samples (bottom panel).
Supplementary figure 5: Subcellular localization of various EGFR-mutation constructs. A) Representative images showing predominant membrane localization of EGFRwt and cytoplasmic localization of EGFRVIII. B) Quantification of images in A. Images were taken at 40x magnification.
Supplementary figure 6: EGFR activation is associated with antibody internalization. A) EGFR activation by various stimuli (EGF, HBEGF, BTC, EPN and AREG, lower panels: unstimulated) results in the internalization of EGFR (number of EGFR-positive vesicles/endosomes) regardless of the presence of blocking antibodies (ABT806 or cetuximab/erbixtux) and type of stimulus. Internalization and activation can be blocked by type I (erlotinib) and type II (lapatinib) inhibitors. B) to demonstrate antibody uptake, we incubated live cells with cetuximab for 2 hr, and after fixation, we stained for cetuximab using only a secondary antibody (green). EGFR counterstaining is shown in red. Unstimulated (starved) cells predominantly show extracellular localization (left). After EGF stimulation, an increase in the number of EGFR-positive vesicles (endosomes) is seen, all of which stain positive for cetuximab. C) Quantification of images in B. As can be seen, EGF stimulation results in an increase in EGFR-positive vesicles in EGFRwt, EGFR<sub>R108K</sub> and EGFR<sub>G598V</sub> and these spots stain positive for cetuximab (Y-axis) confirming increased uptake. Images were taken at 40x magnification.
Supplementary figure 7: mutations in PTEN are associated with response to depatux-m + TMZ. Top: lollipops showing hotspot mutations in PTEN. Bottom, survival curves of samples containing biallelic inactivation of PTEN. Individual variants are listed in table 2.
Supplementary figure 8: mutations in ARID1A, DHFR and RP11.770j4.1 are associated with response to depatux-m + TMZ. Top: lollipop showing hotspot mutations in ARID1A. Bottom, survival curves of samples containing SNVs and LOH of ARID1A (individual variants are listed in table 2), SNVs in DHFR and SNVs in RP11.770j4.1.
Supplementary figure 9: A) Coexpression of genes associated with survival in each of the treatment arms of the study. B) pathway analysis of genes associated with survival. C) NDRG2 was specifically associated with survival in the depatux-m + TMZ arm. Such association was absent in the other two arms of the study.
Supplementary figure 10: VST normalized expression of CDK4 (left) and FOXF1 (right) in relation to overall survival (y-axis). Red dots depict short survivors (< 1y) and in black long survivors (>= 1y).
| EGFR mutation | Treatment | HR    | 95% CI |
|---------------|-----------|-------|--------|
| any mutation  | TMZ|CCNU   | 0.751 | 0.444 | 1.272 |
|               | ABT414   | 0.495 | 0.283 | 0.865 |
|               | TMZ+ABT414 | 0.1058 | 0.530 | 2.113 |
|               | TMZ+ABT414 | 0.510 | 0.247 | 1.055 |
| hotspot mutation | TMZ|CCNU | 1.058 | 0.530 | 2.113 |
|               | ABT414   | 0.799 | 0.313 | 2.037 |
|               | TMZ+ABT414 | 0.386 | 0.138 | 1.082 |
| A289          | TMZ|CCNU   | 0.799 | 0.313 | 2.037 |
|               | ABT414   | 0.386 | 0.138 | 1.082 |
|               | TMZ+ABT414 | 0.254 | 0.027 | 2.370 |
| G598          | TMZ|CCNU   | 1.814 | 0.325 | 10.120 |
|               | ABT414   | 1.076 | 0.207 | 5.590 |
|               | TMZ+ABT414 | 1.004 | 0.570 | 1.771 |
|               | TMZ+ABT414 | 0.582 | 0.311 | 1.088 |
| R108          | TMZ|CCNU   | 0.894 | 0.553 | 1.446 |
|               | ABT414   | 0.673 | 0.412 | 1.099 |
|               | TMZ+ABT414 | 0.285 | 0.087 | 0.937 |
|               | TMZ+ABT414 | 0.255 | 0.077 | 0.846 |
| EGFRvIII absent | TMZ|CCNU | 1.691 | 0.399 | 7.166 |
|               | ABT414   | 0.629 | 0.104 | 3.804 |
|               | TMZ+ABT414 | 2.145 | 0.117 | 39.265 |
|               | TMZ+ABT414 | 0.351 | 0.031 | 3.978 |
| EGFRvIII present | TMZ|CCNU | 1.414 | 0.085 | 23.570 |
|               | ABT414   | 0.000 | 0.000 | Inf   |
|               | TMZ+ABT414 | 0.107 | 0.009 | 1.278 |
|               | TMZ+ABT414 | 0.077 | 0.005 | 1.192 |
| ∆ex25-27      | TMZ|CCNU   | 0.514 | 0.229 | 1.155 |
|               | ABT414   | 0.309 | 0.130 | 0.735 |
|               | TMZ+ABT414 | 0.480 | 0.181 | 1.271 |
|               | TMZ+ABT414 | 0.175 | 0.054 | 0.574 |
| Pr(>|z|) | n  | events | survival (months) |
|---------|----|--------|-------------------|
| 0.287   | 28 | 26     | 6.8               |
|         | 37 | 31     | 8.4               |
| 0.014   | 33 | 26     | 11.7              |
| 0.873   | 15 | 14     | 7.1               |
| 0.070   | 21 | 20     | 7.0               |
|         | 21 | 16     | 10.5              |
| 0.638   | 8  | 8      | 7.5               |
| 0.070   | 11 | 11     | 6.2               |
|         | 11 | 8      | 10.4              |
| 0.229   | 4  | 4      | 4.2               |
|         | 1  | 0      | NA                |
|         | 2  | 1      | 7.8               |
| 0.497   | 3  | 2      | 5.2               |
| 0.931   | 5  | 5      | 7.8               |
|         | 6  | 6      | 9.3               |
| 0.988   | 22 | 20     | 9.1               |
| 0.090   | 35 | 30     | 7.9               |
|         | 28 | 21     | 14.1              |
| 0.649   | 43 | 40     | 7.5               |
| 0.114   | 33 | 29     | 7.3               |
|         | 33 | 27     | 9.8               |
| 0.039   | 11 | 11     | 5.1               |
| 0.026   | 6  | 5      | 9.4               |
|         | 5  | 4      | 16.9              |
| 0.476   | 5  | 3      | 8.4               |
| 0.614   | 6  | 5      | 5.5               |
|         | 3  | 2      | 14.4              |
| 0.607   | 2  | 2      | 6.6               |
| 0.398   | 1  | 1      | 3.9               |
|         | 2  | 2      | 14.1              |
| 0.809   | 2  | 2      | 6.8               |
| 0.999   | 1  | 1      | 3.5               |
|         | 3  | 1      | NA                |
| 0.077   | 3  | 3      | 5.1               |
| 0.067   | 4  | 2      | 17.8              |
|         | 3  | 1      | NA                |
| 0.107   | 16 | 14     | 5.1               |
| 0.008   | 15 | 12     | 8.5               |
|         | 13 | 9      | 16.9              |
| 0.140   | 13 | 13     | 5.1               |
| 0.004   | 8  | 7      | 8.8               |
|         | 7  | 4      | 18.3              |
| EGFR mutation/Gene | Treatment | HR  | 95% CI   | Pr(>|z|) | n  |
|--------------------|-----------|-----|----------|----------|----|
| any mutation       | TMZ|CCNU   | 0.751 | 0.444 1.272 | 0.287 | 28 |
|                    | ABT414    | 0.495 | 0.283 0.865 | 0.014 | 37 |
|                    | TMZ+ABT414| 1.058 | 0.530 2.113 | 0.873 | 15 |
|                    | ABT414    | 0.510 | 0.247 1.055 | 0.070 | 21 |
| hotspot mutation   | TMZ|CCNU   | 1.058 | 0.530 2.113 | 0.873 | 15 |
|                    | ABT414    | 0.510 | 0.247 1.055 | 0.070 | 21 |
|                    | TMZ+ABT414| 0.495 | 0.283 0.865 | 0.014 | 33 |
| A289               | TMZ|CCNU   | 0.799 | 0.313 2.037 | 0.638 | 8  |
|                    | ABT414    | 0.386 | 0.138 1.082 | 0.070 | 11 |
|                    | TMZ+ABT414| 0.254 | 0.027 2.370 | 0.229 | 11 |
| G598               | TMZ|CCNU   | 1.814 | 0.325 10.120 | 0.497 | 9  |
|                    | ABT414    | 1.076 | 0.207 5.590 | 0.931 | 11 |
|                    | TMZ+ABT414| 0.285 | 0.087 0.937 | 0.039 | 6  |
| R108               | TMZ|CCNU   | 1.044 | 0.570 1.771 | 0.988 | 22 |
|                    | ABT414    | 0.582 | 0.311 1.088 | 0.090 | 35 |
|                    | TMZ+ABT414| 0.285 | 0.087 0.937 | 0.039 | 6  |
| EGFRvIII absent    | TMZ|CCNU   | 0.894 | 0.553 1.446 | 0.649 | 33 |
|                    | ABT414    | 0.673 | 0.412 1.099 | 0.114 | 33 |
|                    | TMZ+ABT414| 0.254 | 0.027 2.370 | 0.229 | 11 |
| EGFRvIII present   | TMZ|CCNU   | 0.914 | 0.570 1.446 | 0.649 | 33 |
|                    | ABT414    | 0.673 | 0.412 1.099 | 0.114 | 33 |
|                    | TMZ+ABT414| 0.254 | 0.027 2.370 | 0.229 | 11 |
| ∆ex25-27           | TMZ|CCNU   | 0.285 | 0.087 0.937 | 0.039 | 6  |
|                    | ABT414    | 0.255 | 0.077 0.846 | 0.026 | 5  |
|                    | TMZ+ABT414| 0.254 | 0.027 2.370 | 0.229 | 11 |
| ∆ex25-26           | TMZ|CCNU   | 1.691 | 0.399 7.166 | 0.476 | 6  |
|                    | ABT414    | 0.629 | 0.104 3.804 | 0.614 | 3  |
|                    | TMZ+ABT414| 0.255 | 0.077 0.846 | 0.026 | 5  |
| ∆ex27              | TMZ|CCNU   | 2.145 | 0.117 39.265 | 0.607 | 2  |
|                    | ABT414    | 0.351 | 0.031 3.978 | 0.398 | 2  |
|                    | TMZ+ABT414| 0.255 | 0.077 0.846 | 0.026 | 5  |
| C-term del         | TMZ|CCNU   | 1.414 | 0.085 23.570 | 0.809 | 2  |
|                    | ABT414    | 0.351 | 0.031 3.978 | 0.398 | 2  |
|                    | TMZ+ABT414| 0.000 | 0.000 Inf    | 0.999 | 3  |
| C-term SNV         | TMZ|CCNU   | 0.107 | 0.009 1.278 | 0.077 | 3  |
|                    | ABT414    | 0.077 | 0.005 1.192 | 0.067 | 4  |
|                    | TMZ+ABT414| 0.000 | 0.000 Inf    | 0.999 | 3  |
| all-C-term         | TMZ|CCNU   | 0.514 | 0.229 1.155 | 0.107 | 16 |
|                    | ABT414    | 0.309 | 0.130 0.735 | 0.008 | 15 |
|                    | TMZ+ABT414| 0.175 | 0.054 0.574 | 0.004 | 13 |
| all-C-term trunc   | TMZ|CCNU   | 0.480 | 0.181 1.271 | 0.140 | 8  |
|                    | ABT414    | 0.175 | 0.054 0.574 | 0.004 | 7  |
|                    | TMZ+ABT414| 0.175 | 0.054 0.574 | 0.004 | 7  |

| PTEN all | TMZ|CCNU | 16 |
| Gene    | TMZ| CCNU | ABT414 | TMZ+ABT414 |
|---------|----|------|--------|------------|
| PTEN HD | TMZ| CCNU | 1.291  | 0.392      |
|         | ABT414 | 0.540 | 0.125  | 0.475      |
|         | TMZ+ABT414 | 3.088 | 1.229  | 0.914      | 14  |
| PTEN SNV| TMZ| CCNU | 0.876  | 0.659      |
|         | ABT414 | 0.636 | 0.475  | 0.914      | 18  |
|         | TMZ+ABT414 | 1.207 | 0.417  | 0.013      | 15  |
| ARID1A SNV| TMZ| CCNU | 0.541  | 0.139      |
|         | ABT414 | 0.086 | 0.015  | 0.125      | 10  |
|         | TMZ+ABT414 | 3.410 | 1.258  | 0.079      | 15  |
| ARID1A LOH| TMZ| CCNU | 0.286  | 0.269      |
|         | ABT414 | 0.047 | 0.075  | 0.961      | 21  |
|         | TMZ+ABT414 | 1.745 | 0.395  | 0.043      | 15  |
| ARID1A SNV+LOH| TMZ| CCNU | 0.572  | 0.322      |
|         | ABT414 | 0.158 | 0.100  | 1.036      | 27  |
|         | TMZ+ABT414 | 2.070 | 1.063  | 0.057      | 15  |
| RP11.770J1.4| TMZ| CCNU | 0.831  | 0.322      |
|         | ABT414 | 0.379 | 0.100  | 1.001      | 28  |
|         | TMZ+ABT414 | 1.821 | 1.036  | 0.057      | 15  |
| DHFR    | TMZ| CCNU | 1.003  | 0.587      |
|         | ABT414 | 0.599 | 0.345  | 1.001      | 36  |
|         | TMZ+ABT414 | 1.677 | 0.992  | 0.050      | 36  |
| Events | Survival (months) |
|--------|------------------|
| 26     | 6.8              |
| 31     | 8.4              |
| 26     | 11.7             |
| 14     | 7.1              |
| 20     | 7.0              |
| 16     | 10.5             |
| 8      | 7.5              |
| 11     | 6.2              |
| 8      | 10.4             |
| 4      | 4.2              |
| 0      | NA               |
| 1      | 7.8              |
| 2      | 5.2              |
| 5      | 7.8              |
| 6      | 9.3              |
| 20     | 9.1              |
| 30     | 7.9              |
| 21     | 14.1             |
| 40     | 7.5              |
| 29     | 7.3              |
| 27     | 9.8              |
| 11     | 5.1              |
| 5      | 9.4              |
| 4      | 16.9             |
| 3      | 8.4              |
| 5      | 5.5              |
| 2      | 14.4             |
| 2      | 6.6              |
| 1      | 3.9              |
| 2      | 14.1             |
| 2      | 6.8              |
| 1      | 3.5              |
| 1      | NA               |
| 3      | 5.1              |
| 2      | 17.8             |
| 1      | NA               |
| 14     | 5.1              |
| 12     | 8.5              |
| 9      | 16.9             |
| 13     | 5.1              |
| 7      | 8.8              |
| 4      | 18.3             |
| 16     | 8.4              |
|   |     |
|---|-----|
| 19| 8.4 |
| 15|10.2 |
| 13| 5.4 |
|  9| 5.5 |
|  6| 8.2 |
| 13| 8.8 |
| 15| 8.7 |
| 11|11.0 |
|  2| 2.6 |
|  5| 5.4 |
|  3|16.6 |
|  3| 10.5 |
|  0|  NA |
|  5|15.1 |
|  5| 4.2 |
|  5| 8.2 |
|  8|15.4 |
| 10| 6.7 |
| 17| 8.4 |
|  4|14.8 |
| 27| 9.5 |
| 32| 7.9 |
| 28|11.7 |
| Gene          | Treatment     | HR   | 95% CI    | Pr(>|z|) | n  | events | survival (months) |
|--------------|---------------|------|-----------|---------|----|--------|------------------|
| PTEN all     | TMZ | CCNU      | 0.692 | 0.353 | 1.353 | 0.282 | 16 | 16 | 8.4 |
|              | ABT414        | 0.499 | 0.241 | 1.034 | 0.061 |        | 23 | 19 | 8.4 |
|              | TMZ+ABT414    |       |        |        |       |        | 20 | 15 | 10.2 |
| PTEN HD      | TMZ | CCNU      | 1.291 | 0.540 | 3.088 | 0.566 | 14 | 13 | 5.4 |
|              | ABT414        | 0.392 | 0.125 | 1.229 | 0.108 |        | 9  | 9  | 5.5 |
|              | TMZ+ABT414    |       |        |        |       |        | 7  | 6  | 8.2 |
| PTEN SNV     | TMZ | CCNU      | 0.876 | 0.636 | 1.207 | 0.417 | 13 | 13 | 8.8 |
|              | ABT414        | 0.659 | 0.475 | 0.914 | 0.013 |        | 18 | 15 | 8.7 |
|              | TMZ+ABT414    |       |        |        |       |        | 15 | 11 | 11.0 |
| ARID1A SNV   | TMZ | CCNU      | 0.541 | 0.086 | 3.410 | 0.513 | 2  | 2  | 2.6 |
|              | ABT414        | 0.139 | 0.015 | 1.258 | 0.079 |        | 5  | 5  | 5.4 |
|              | TMZ+ABT414    |       |        |        |       |        | 5  | 3  | 16.6 |
| ARID1A LOH   | TMZ | CCNU      | 0.286 | 0.047 | 1.745 | 0.175 | 3  | 3  | 10.5 |
|              | ABT414        |        |        |        |       |        | 1  | 0  | NA  |
|              | TMZ+ABT414    |       |        |        |       |        | 6  | 5  | 15.1 |
| AIRD1A SNV+LOH| TMZ | CCNU      | 0.572 | 0.158 | 2.070 | 0.395 | 5  | 5  | 4.2 |
|              | ABT414        | 0.269 | 0.075 | 0.961 | 0.043 |        | 6  | 5  | 8.2 |
|              | TMZ+ABT414    |       |        |        |       |        | 11 | 8  | 15.4 |
| RP11.770J1.4 | TMZ | CCNU      | 0.831 | 0.379 | 1.821 | 0.643 | 10 | 10 | 6.7 |
|              | ABT414        | 0.322 | 0.100 | 1.036 | 0.057 |        | 19 | 17 | 8.4 |
|              | TMZ+ABT414    |       |        |        |       |        | 8  | 4  | 14.8 |
| DHFR         | TMZ | CCNU      | 1.003 | 0.599 | 1.677 | 0.992 | 28 | 27 | 9.5 |
|              | ABT414        | 0.587 | 0.345 | 1.001 | 0.050 |        | 36 | 32 | 7.9 |
|              | TMZ+ABT414    |       |        |        |       |        | 36 | 28 | 11.7 |
|                        |         |         |         |
|------------------------|---------|---------|---------|
| **EGFR SNV present**   |         |         |         |
|                        | HR      | 95% CI  |         |
| ABT414                 | 0.718   | 0.430   | 1.199   |
| TMZ+ABT414             | 0.448   | 0.263   | 0.760   |
| MGMT Methylated        | 0.447   | 0.285   | 0.702   |
| Age >40, <60           | 1.128   | 0.316   | 2.488   |
| Age >60                | 1.434   | 0.508   | 4.053   |
| **EGFRvIII absent**    |         |         |         |
|                        | HR      | 95% CI  |         |
| ABT414                 | 1.096   | 0.601   | 1.999   |
| TMZ+ABT414             | 0.566   | 0.310   | 1.033   |
| MGMT Methylated        | 0.519   | 0.315   | 0.853   |
| Age >40, <60           | 1.030   | 0.308   | 3.443   |
| Age >60                | 1.62568 | 0.4952  | 5.337   |
| p   |       |
|-----|-------|
| 0.206 | 0.003 |
| 0.000 | 0.819 |
| 0.496 |       |
| p   |       |
| 0.764 | 0.064 |
| 0.010 | 0.962 |
| 0.423 |       |
| PATID | sex | treatment arm | censor | OS (months) | COSMIC_ID   | variantFreq |
|-------|-----|---------------|--------|-------------|-------------|-------------|
| 68    | F   | ABT414        | 1      | 19.0        | COSM1675407 | 0.721       |
| 88    | M   | TMZ+ABT414    | 1      | 3.1         | COSM5027    | 0.886       |
| 107   | M   | TMZ+ABT414    | 1      | 3.7         | COSM28917   | 0.534       |
| 124   | M   | TMZ+ABT414    | 1      | 1.1         |             | 0.738       |
| 149   | F   | TMZ|CCNU         | 1      | 4.2         | COSM4896    | 0.736       |
| 178   | F   | ABT414        | 1      | 3.8         | COSM5109    | 0.677       |
| 188   | M   | ABT414        | 0      | 18.7        |             | 0.368       |
| 191   | F   | TMZ|CCNU         | 1      | 19.1        | COSM23643   | 0.468       |
| 191   | F   | TMZ|CCNU         | 1      | 19.1        | COSM30611   | 0.381       |
| 191   | F   | TMZ|CCNU         | 1      | 19.1        | COSM23643   | 0.422       |
| 267   | M   | ABT414        | 1      | 3.4         | COSM13459   | 0.722       |
| 295   | M   | TMZ|CCNU         | 1      | 2.9         | COSM249839  | 0.762       |
| 299   | M   | TMZ+ABT414    | 1      | 9.4         | COSM1173628 | 0.375       |
| 321   | M   | TMZ+ABT414    | 1      | 24.1        |             | 0.503       |
| 322   | F   | TMZ+ABT414    | 1      | 6.0         | COSM23566   | 0.843       |
| 323   | M   | TMZ|CCNU         | 1      | 8.9         | COSM4894833 | 0.609       |
| 330   | M   | TMZ|CCNU         | 1      | 5.2         | COSM4995    | 0.602       |
| 340   | M   | ABT414        | 1      | 14.3        | COSM1627662 | 0.222       |
| 361   | M   | ABT414        | 1      | 7.1         | COSM5347060 | 0.654       |
| 375   | M   | ABT414        | 0      | 18.2        | COSM5033    | 0.816       |
| 375   | M   | ABT414        | 0      | 18.2        | COSM5033    | 0.288       |
| 391   | M   | TMZ|CCNU         | 1      | 8.0         | COSM249825  | 0.728       |
| 396   | F   | ABT414        | 1      | 8.4         | COSM5347090 | 0.226       |
| 418   | M   | TMZ+ABT414    | 1      | 6.8         | COSM5151    | 0.159       |
| 435   | M   | TMZ|CCNU         | 1      | 13.9        | COSM5160    | 0.704       |
| 447   | M   | TMZ+ABT414    | 1      | 7.4         | COSM5157    | 0.759       |
| 454   | M   | TMZ|CCNU         | 1      | 8.8         | COSM921142  | 0.629       |
| 457   | M   | TMZ+ABT414    | 0      | 10.6        | COSM1666994 | 0.747       |
| 490   | M   | ABT414        | 1      | 3.0         | COSM1173628 | 0.587       |
| 512   | M   | TMZ+ABT414    | 1      | 4.5         | COSM4898    | 0.630       |
| 538   | F   | TMZ|CCNU         | 1      | 6.5         | COSM1666994 | 0.467       |
| 555   | F   | ABT414        | 1      | 5.9         | COSM4936    | 0.652       |
| 576   | M   | ABT414        | 1      | 1.9         | COSM921127  | 0.399       |
| 594   | M   | TMZ+ABT414    | 1      | 18.3        | COSM4936    | 0.623       |
| 631   | M   | TMZ|CCNU         | 1      | 9.6         | COSM428079  | 0.273       |
| 636   | M   | ABT414        | 1      | 17.8        | COSM13578   | 0.445       |
| 649   | M   | ABT414        | 1      | 5.5         | COSM1744939 | 0.891       |
| 657   | F   | ABT414        | 1      | 1.2         | COSM4963    | 0.569       |
| 695   | M   | TMZ+ABT414    | 0      | 20.6        | COSM1173647 | 0.232       |
| 696   | M   | TMZ+ABT414    | 0      | 20.3        | COSM5295    | 0.534       |
| 730   | F   | ABT414        | 0      | 21.0        | COSM23654   | 0.631       |
| 768   | M   | ABT414        | 0      | 19.5        | COSM5090    | 0.620       |
| 786   | M   | ABT414        | 1      | 7.8         | COSM5151    | 0.455       |
| 791   | F   | TMZ+ABT414    | 0      | 19.2        | COSM5033    | 0.533       |
| 816   | F   | TMZ+ABT414    | 1      | 5.4         | COSM1173628 | 0.731       |
| 867   | M   | TMZ+ABT414    | 1      | 4.1         | COSM5163    | 0.740       |
| 869   | M   | TMZ+ABT414    | 0      | 19.3        |             | 0.609       |
| 892   | M   | TMZ|CCNU         | 1      | 9.4         | COSM28884   | 0.399       |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
|   | F | TMZ+ABT414 | 1 | 8.9 | COSM5082 | 0.381 |
|   | M | ABT414 | 1 | 0.2 |   | 0.699 |
| 962 | M | TMZ|CCNU | 1 | 10.2 | COSM4408405 | 0.479 |
| 974 | M | TMZ|CCNU | 1 | 7.0 | COSM35849 | 0.729 |
| 999 | M | TMZ+ABT414 | 1 | 2.2 | COSM5313 | 0.546 |
| 1017 | M | TMZ+ABT414 | 1 | 11.0 | COSM5103 | 0.701 |
| 1032 | M | TMZ+ABT414 | 0 | 18.4 | COSM5039 | 0.612 |
| 1057 | M | TMZ+ABT414 | 0 | 17.9 | COSM3736942 | 0.192 |
| 1062 | M | TMZ+ABT414 | 1 | 6.1 | COSM27635 | 0.418 |
| 1065 | F | ABT414 | 1 | 4.2 | COSM5291 | 0.734 |
| 1077 | M | ABT414 | 1 | 10.5 | COSM1744939 | 0.696 |
| 1083 | M | ABT414 | 1 | 9.0 | COSM5105 | 0.536 |
| 1096 | M | TMZ+ABT414 | 1 | 17.3 | COSM428084 | 0.358 |
| 1099 | F | TMZ+ABT414 | 1 | 2.0 | COSM5123 | 0.828 |
| 1110 | M | ABT414 | 1 | 13.0 | COSM308347 | 0.363 |
| 1120 | M | TMZ+ABT414 | 1 | 15.4 | COSM921068 | 0.463 |
| 1128 | F | TMZ+ABT414 | 1 | 6.3 | COSM1173628 | 0.902 |
| consequence | Mutation                        |
|-------------|--------------------------------|
| missense_variant | p.Tyr46Cys                   |
| missense_variant | p.Asp162Glu                   |
| missense_variant | p.Gly129Glu                   |
| frameshift_variant | p.Ile101SerfsTer12         |
| frameshift_variant | p.Thr319LysfsTer24         |
| missense_variant | p.Ile101Thr                   |
| missense_variant | p.Arg159Gly                   |
| frameshift_variant | p.Glu106Ter                   |
| missense_variant | p.Asp107Tyr                   |
| stop_gained   | p.CysGlu105TerGln            |
| frameshift_variant | p.Ser113ArgfsTer2          |
| missense_variant | p.Tyr68Asn                   |
| stop_gained   | p.Arg233Ter                   |
| missense_variant | p.Gly209Glu                   |
| missense_variant | p.Asp92Tyr                   |
| frameshift_variant | p.Glu43LysfsTer11         |
| missense_variant | p.Cys136Arg                   |
| frameshift_variant | p.Cys296Ter                   |
| missense_variant | p.Phe195Ser                   |
| missense_variant | p.Arg130Gln                   |
| frameshift_variant | p.Arg130GlnfsTer4          |
| stop_gained   | p.Gly127Ter                   |
| missense_variant | p.His259Pro                   |
| stop_gained   | p.Arg335Ter                   |
| stop_gained   | p.Gln261Ter                   |
| missense_variant | p.Trp111Ser                   |
| frameshift_variant | p.Ile300MetfsTer7          |
| missense_variant | p.Met35Val                   |
| stop_gained   | p.Arg233Ter                   |
| frameshift_variant | p.Thr319Ter                   |
| missense_variant | p.Met35Val                   |
| frameshift_variant | p.Phe347SerfsTer5          |
| frameshift_variant | p.Pro248ThrfsTer5          |
| frameshift_variant | p.Phe347TyrfsTer13         |
| missense_variant | p.Arg47Lys                   |
| missense_variant | p.Cys124Tyr                   |
| frameshift_variant | p.Asn329LysfsTer14          |
| missense_variant | p.Tyr76Asn                   |
| missense_variant | p.Pro246Leu                   |
| stop_gained   | p.Ser229Ter                   |
| frameshift_variant | p.Glu235LysfsTer21         |
| missense_variant | p.Pro96Leu                   |
| stop_gained   | p.Arg335Ter                   |
| missense_variant | p.Arg130Gln                   |
| stop_gained   | p.Arg233Ter                   |
| stop_gained   | p.Gln87Ter                   |
| frameshift_variant | p.Asn311Ter                   |
| missense_variant | p.Ala126Gly                   |
| Type                      | Variant       |
|---------------------------|---------------|
| missense_variant          | p.Lys125Gln   |
| missense_variant          | p.Gly209Glu   |
| missense_variant          | p.Ile33Thr    |
| frameshift_variant        | p.Glu242Ter   |
| stop_gained               | p.Ser59Ter    |
| missense_variant          | p.Gly156Arg   |
| missense_variant          | p.Arg173His   |
| missense_variant          | p.Leu247Ser   |
| missense_variant          | p.Phe341Cys   |
| stop_gained               | p.Tyr225Ter   |
| frameshift_variant        | p.Asn329LysfsTer14 |
| missense_variant          | p.Pro95Leu    |
| stop_gained               | p.Tyr88Ter    |
| missense_variant          | p.Gly132Asp   |
| stop_gained               | p.Arg130Ter   |
| missense_variant          | p.Asp52Val    |
| stop_gained               | p.Arg233Ter   |
|                | HR   | 95% CI | p     |
|----------------|------|--------|-------|
| ABT414         | 0.867| 0.601  | 1.252 | 0.447 |
| TMZ+ABT414     | 0.603| 0.416  | 0.875 | 0.008 |
| MGMT Methylated| 0.550| 0.402  | 0.752 | 0.000 |
| PTEN LOH       | 0.874| 0.422  | 1.809 | 0.717 |
| PTEN wt        | 0.749| 0.289  | 1.939 | 0.552 |
|                |      |        |       |
| ABT414         | 0.872| 0.600  | 1.266 | 0.470 |
| TMZ+ABT414     | 0.615| 0.419  | 0.903 | 0.013 |
| MGMT Methylated| 0.534| 0.390  | 0.732 | 0.000 |
| TP53 mut       | 1.561| 1.038  | 2.346 | 0.032 |
| gene.id   | Beta    | Z       | P      | Wald   | HR     |
|----------|---------|---------|--------|--------|--------|
| AC078906.1| 1.4571  | 4.0197  | 0.0001 | 0.0001 | 4.2933 |
| DLGAP1| 0.4927  | 4.0999  | 0      | 0      | 1.6368 |
| TIAM2| 0.8737  | 4.0329  | 0.0001 | 0.0001 | 2.3957 |
| MIR3691| 1.8868  | 3.8936  | 0.0001 | 0.0001 | 6.5985 |
| AC026358.1| 0.9745  | 3.8199  | 0.0001 | 0.0001 | 2.6498 |
| ZNF518B| -0.447  | -3.6262 | 0.0003 | 0.0003 | 0.6395 |
| CACNG4| 0.3723  | 3.5885  | 0.0003 | 0.0003 | 1.4511 |
| THAP12P2| 0.6191  | 3.573   | 0.0004 | 0.0004 | 1.8573 |
| KLF11| -0.7748 | -3.5452 | 0.0004 | 0.0004 | 0.4608 |
| AC092111.1| 0.7364  | 3.4893  | 0.0005 | 0.0005 | 2.0884 |
| AC078842.1| 0.8516  | 3.5559  | 0.0004 | 0.0004 | 2.3433 |
| MPPED2| 0.4112  | 3.4334  | 0.0006 | 0.0006 | 1.5078 |
| LINGO1-AS1| 0.8874  | 3.4435  | 0.0006 | 0.0006 | 2.4288 |
| PLPPR5| 0.4524  | 3.3994  | 0.0007 | 0.0007 | 1.572  |
| CHRNA6| -0.9506 | -3.3905 | 0.0007 | 0.0007 | 0.3865 |
| AC022121.1| 1.0861  | 3.4171  | 0.0006 | 0.0006 | 2.9627 |
| KCNM8| -0.7434 | -3.4392 | 0.0006 | 0.0006 | 0.4755 |
| SRSF12| 0.6848  | 3.4171  | 0.0006 | 0.0006 | 1.9833 |
| TNFSF10| -0.6256 | -3.4294 | 0.0006 | 0.0006 | 0.5349 |
| TRIM54| 0.841   | 3.4064  | 0.0007 | 0.0007 | 2.3188 |
| ZNF485| -1.1614 | -3.3717 | 0.0007 | 0.0007 | 0.3131 |
| HPX| 0.8625  | 3.3836  | 0.0007 | 0.0007 | 2.3691 |
| P2RY4| 1.0243  | 3.3601  | 0.0008 | 0.0008 | 2.785  |
| AFDN| 0.9076  | 3.3759  | 0.0007 | 0.0007 | 2.4785 |
| ST8SIA5| 0.2713  | 3.3194  | 0.0009 | 0.0009 | 1.3116 |
| AC027801.1| 0.5133  | 3.3209  | 0.0009 | 0.0009 | 1.6707 |
| AC124947.1| -1.8773 | -3.2929 | 0.001  | 0.001  | 0.153  |
| GREB1| 0.49    | 3.3113  | 0.0009 | 0.0009 | 1.6323 |
| DISP2| 0.4255  | 3.3284  | 0.0009 | 0.0009 | 1.5303 |
| AF121898.1| 1.3601  | 3.2639  | 0.0011 | 0.0011 | 3.8966 |
| GAD1| 0.25    | 3.2604  | 0.0011 | 0.0011 | 1.284  |
| OACYLP| 0.6397  | 3.2863  | 0.001  | 0.001  | 1.8959 |
| ADAMTS7P4| 0.6119  | 3.3071  | 0.0009 | 0.0009 | 1.8439 |
| BMS1P2| 0.9866  | 3.3293  | 0.0009 | 0.0009 | 2.6821 |
| LINCO0475| -0.4167 | -3.2173 | 0.0013 | 0.0013 | 0.6592 |
| ZNF662| 0.849   | 3.2276  | 0.0012 | 0.0012 | 2.3372 |
| AC108215.1| 0.3885  | 3.2032  | 0.0014 | 0.0014 | 1.4747 |
| HEG1| -0.9015 | -3.2191 | 0.0013 | 0.0013 | 0.406  |
| NCAPD3| 1.4635  | 3.2152  | 0.0013 | 0.0013 | 4.3212 |
| C2orf80| 0.4878  | 3.2069  | 0.0013 | 0.0013 | 1.6288 |
| ACSL5| -0.6688 | -3.1932 | 0.0014 | 0.0014 | 0.5123 |
| DRD2| 0.4139  | 3.1899  | 0.0014 | 0.0014 | 1.5127 |
| AP004608.1| 0.6228  | 3.2575  | 0.0011 | 0.0011 | 1.8642 |
| TTC24| 0.6928  | 3.1743  | 0.0015 | 0.0015 | 1.9993 |
| HS6ST2| 0.332   | 3.175   | 0.0015 | 0.0015 | 1.3937 |
| ABI3BP| -0.274  | -3.1476 | 0.0016 | 0.0016 | 0.7603 |
| HAPLN1| 0.3172  | 3.1293  | 0.0018 | 0.0018 | 1.3732 |
| Gene   | Chromosome | Position | p-value  | p-value  | Size  |
|--------|------------|----------|----------|----------|-------|
| TRMT9B | chr8:13M   | 0.4713   | 3.1394   | 0.0017   | 0.0017 | 1.6021 |
| COL28A1| chr7:7M    | 0.2533   | 3.119    | 0.0018   | 0.0018 | 1.2882 |
| ZNF433 | chr19:12M  | 0.8285   | 3.1524   | 0.0016   | 0.0016 | 2.2898 |
Genes associated with survival in the depatux-m monotherapy arm

| gene.id       | Beta  | Z      | P      | Wald  | HR    |
|---------------|-------|--------|--------|-------|-------|
| TRIM22|chr11:6M | -1.0041 | -4.0092 | 0.0001 | 0.0001 | 0.3664 |
| BANK1|chr4:102M | -0.9445 | -4.0117 | 0.0001 | 0.0001 | 0.3889 |
| AC115522.1|chr19:44M | 1.6241 | 3.8821 | 0.0001 | 0.0001 | 5.074  |
| VNN1|chr6:133M | -1.5262 | -4.0537 | 0.0001 | 0.0001 | 0.2174 |
| XAF1|chr17:7M | -0.4941 | -3.7976 | 0.0001 | 0.0001 | 0.6101 |
| ZFHX3|chr16:73M | -1.5297 | -3.828  | 0.0001 | 0.0001 | 0.2166 |
| PITHD1|chr1:24M | 1.4973 | 3.6849 | 0.0002 | 0.0002 | 4.4695 |
| OAS1|chr12:113M | -0.4908 | -3.5998 | 0.0003 | 0.0003 | 0.6121 |
| AL162431.1|chr12:110M | -1.4981 | -3.5314 | 0.0004 | 0.0004 | 0.2235 |
| UBE3B|chr12:110M | -2.5423 | -3.517  | 0.0004 | 0.0004 | 0.0787 |
| LBR|chr1:225M | 0.9501 | 3.5107 | 0.0004 | 0.0004 | 2.586  |
| KIAA1671|chr22:25M | -1.0162 | -3.497  | 0.0005 | 0.0005 | 0.362  |
| EMC1|chr1:19M | 1.4416 | 3.5236 | 0.0004 | 0.0004 | 4.2275 |
| ATP5MGL|chr22:43M | -1.5804 | -3.4824 | 0.0005 | 0.0005 | 0.2059 |
| FYCO1|chr3:46M | -1.1549 | -3.465  | 0.0005 | 0.0005 | 0.3151 |
| TF2|chr7:101M | 0.531  | 3.4423 | 0.0006 | 0.0006 | 1.7005 |
| TMRPSS2|chr21:41M | -0.7576 | -3.3367 | 0.0008 | 0.0008 | 0.4688 |
| TMEM221|chr19:17M | -0.6403 | -3.4369 | 0.0006 | 0.0006 | 0.5272 |
| SSTR3|chr22:37M | 0.8569 | 3.3997 | 0.0007 | 0.0007 | 2.3557 |
| MYBPHL|chr1:109M | 0.9547 | 3.473  | 0.0005 | 0.0005 | 2.5978 |
| UGGT2|chr13:96M | 0.8662 | 3.4205 | 0.0006 | 0.0006 | 2.3778 |
| RNF219|chr13:79M | 0.904  | 3.4486 | 0.0006 | 0.0006 | 2.4695 |
| ZDHC2|chr8:17M | -0.7237 | -3.4311 | 0.0006 | 0.0006 | 0.485  |
| AC009831.1|chr18:32M | -1.426 | -3.4312 | 0.0006 | 0.0006 | 0.2403 |
| PYCR1|chr17:82M | 0.6483 | 3.3838 | 0.0007 | 0.0007 | 1.9123 |
| APOBEC3F|chr22:39M | -0.721 | -3.3344 | 0.0009 | 0.0009 | 0.4863 |
| GIMAP8|chr7:150M | -0.8466 | -3.3177 | 0.0009 | 0.0009 | 0.4289 |
| TNFSF10|chr3:173M | -0.6548 | -3.2858 | 0.001  | 0.001  | 0.5195 |
| IPO5|chr13:98M | 1.1989 | 3.279  | 0.001  | 0.001  | 3.3166 |
| RFO0091|chr4:68M | 0.7265 | 3.2461 | 0.0012 | 0.0012 | 2.0679 |
| UNC5A|chr5:177M | 0.4481 | 3.2576 | 0.0011 | 0.0011 | 1.5653 |
| TFRC|chr3:196M | 0.7306 | 3.2512 | 0.0011 | 0.0011 | 2.0764 |
| SLC25A15|chr13:41M | 0.9712 | 3.2035 | 0.0014 | 0.0014 | 2.6411 |
| NPAS2|chr2:101M | -0.4377 | -3.2475 | 0.0012 | 0.0012 | 0.6455 |
| KRT8|chr12:53M | -0.9224 | -3.2741 | 0.0011 | 0.0011 | 0.3976 |
| KCNJ4|chr22:38M | 0.476 | 3.2011 | 0.0014 | 0.0014 | 1.6096 |
| FLI1|chr11:129M | -0.8218 | -3.2209 | 0.0013 | 0.0013 | 0.4397 |
| AC091826.2|chr5:88M | 1.027 | 3.2019 | 0.0014 | 0.0014 | 2.7927 |
| MORC4|chrX:107M | -1.2272 | -3.2367 | 0.0012 | 0.0012 | 0.2931 |
| UTRN|chr6:145M | -0.9107 | -3.2264 | 0.0013 | 0.0013 | 0.4023 |
| CCDC69|chr5:151M | -0.6505 | -3.181  | 0.0015 | 0.0015 | 0.5218 |
| EPBH6|chr7:143M | 0.4757 | 3.1677 | 0.0015 | 0.0015 | 1.6092 |
| ADA2|chr22:17M | -0.4813 | -3.1768 | 0.0015 | 0.0015 | 0.618  |
| SNORD91A|chr17:2M | 1.5316 | 3.1813 | 0.0015 | 0.0015 | 4.6255 |
| MAF|chr16:80M | -0.7646 | -3.1995 | 0.0014 | 0.0014 | 0.4655 |
| GBAP1|chr1:155M | 0.9165 | 3.1533 | 0.0016 | 0.0016 | 2.5005 |
| CASP10|chr2:201M | -0.9303 | -3.1604 | 0.0016 | 0.0016 | 0.3944 |
| Gene     | Chromosome | Start (bp) | End (bp) | p-value1 | p-value2 | p-value3 |
|----------|------------|------------|----------|----------|----------|----------|
| LTA4H    | chr12      | 96M        | -1.1694  | -3.1557  | 0.0016   | 0.0016   | 0.3106   |
| RTBDN    | chr19      | 13M        | 1.3057   | 3.112    | 0.0019   | 0.0019   | 3.6901   |
| APOC1P1  | chr19      | 45M        | -1.1163  | -3.1376  | 0.0017   | 0.0017   | 0.3275   |
| gene.id | Beta     | Z       | P        | Wald    | HR      |
|---------|----------|---------|----------|---------|---------|
| YEATS2| chr3:184M | 1.3252 | 3.667 | 0.0002 | 0.0002 | 3.7628 |
| HIST1H4K| chr6:28M | 0.9528 | 3.524 | 0.0004 | 0.0004 | 2.5929 |
| SLC24A4| chr14:92M | -0.3454 | -3.4253 | 0.0006 | 0.0006 | 0.708  |
| FOXN3| chr14:89M | -1.6641 | -3.3232 | 0.0009 | 0.0009 | 0.1894 |
| DNMBP| chr10:100M | 1.024 | 3.2998 | 0.001 | 0.001 | 2.7842 |
| AC005609.5| chr5:141M | -1.2471 | -3.2581 | 0.0011 | 0.0011 | 0.2873 |
| DGLUCY| chr14:91M | -1.3433 | -3.2956 | 0.001 | 0.001 | 0.261  |
| NDRG2| chr14:21M | -0.6052 | -3.2525 | 0.0011 | 0.0011 | 0.546  |
| HEATR4| chr14:74M | -0.6854 | -3.2447 | 0.0012 | 0.0012 | 0.5039 |
| DBF4P1| chr10:64M | 2.1913 | 3.2929 | 0.001 | 0.001 | 8.9471 |
| GHRL| chr3:10M | -1.2013 | -3.2083 | 0.0013 | 0.0013 | 0.3008 |
| PEBP4| chr8:23M | -1.2872 | -3.1392 | 0.0017 | 0.0017 | 0.276  |
| COL4A3| chr2:222M | -0.3841 | -3.1369 | 0.0017 | 0.0017 | 0.681  |
| VMAC| chr19:6M | -1.0008 | -3.0733 | 0.0021 | 0.0021 | 0.3676 |
| GTF2H2B| chr5:70M | 0.5182 | 3.0141 | 0.0026 | 0.0026 | 1.679  |
| LINC01285| chrX:119M | -0.6189 | -3.0685 | 0.0022 | 0.0022 | 0.5385 |
| TTTY14| chrY:19M | 1.0314 | 3.0571 | 0.0022 | 0.0022 | 2.8051 |
| CAVIN2| chr2:192M | -0.4773 | -3.0058 | 0.0026 | 0.0026 | 0.6205 |
| PHF13| chr1:7M | 0.6149 | 2.9892 | 0.0028 | 0.0028 | 1.8494 |
| AL713852.1| chr6:44M | 1.464 | 3.0195 | 0.0025 | 0.0025 | 4.3231 |
| DCHS2| chr4:154M | -0.4987 | -2.9584 | 0.0031 | 0.0031 | 0.6073 |
| EML5| chr14:89M | -0.564 | -2.986 | 0.0028 | 0.0028 | 0.5689 |
| XPO5| chr6:44M | 1.6162 | 2.9655 | 0.003 | 0.003 | 5.034  |
| AL109615.1| chr6:44M | 1.3522 | 2.9455 | 0.0032 | 0.0032 | 3.8658 |
| LINC00628| chr1:204M | 0.9164 | 2.8936 | 0.0038 | 0.0038 | 2.5003 |
| GUSBP9| chr5:71M | 1.3134 | 2.9304 | 0.0034 | 0.0034 | 3.7187 |
| TMEM131L| chr4:154M | 0.6672 | 2.9144 | 0.0036 | 0.0036 | 1.9487 |
| AC006487.2| chr17:50M | -1.1308 | -2.9185 | 0.0035 | 0.0035 | 0.3228 |
| FMO2| chr1:171M | -0.6225 | -2.9264 | 0.0034 | 0.0034 | 0.5366 |
| ZC3H11B| chr1:220M | 1.1361 | 2.9291 | 0.0034 | 0.0034 | 3.1145 |
| PEL1| chr14:56M | -0.7798 | -2.8948 | 0.0038 | 0.0038 | 0.4585 |
| FAM20B| chr1:179M | 1.2708 | 2.881 | 0.004 | 0.004 | 3.5639 |
| NAP1L2| chrX:73M | -0.3979 | -2.8705 | 0.0041 | 0.0041 | 0.6717 |
| GSG1| chr16:28M | -0.3134 | -2.8455 | 0.0044 | 0.0044 | 0.731  |
| HSD17BP2| chr10:38M | 0.7211 | 2.8532 | 0.0043 | 0.0043 | 2.0568 |
| SMIM7| chr19:17M | -1.3593 | -2.8815 | 0.004 | 0.004 | 0.2568 |
| GGT1P| chr9:121M | -0.4849 | -2.8945 | 0.0038 | 0.0038 | 0.6158 |
| TTTY15| chrY:13M | -0.3591 | -2.7624 | 0.0057 | 0.0057 | 0.6983 |
| KLHL31| chr6:54M | -1.0894 | -2.8264 | 0.0047 | 0.0047 | 0.3364 |
| AC093752.3| chr4:119M | -1.0293 | -2.8155 | 0.0049 | 0.0049 | 0.3573 |
| METTL7A| chr12:51M | -0.4612 | -2.8015 | 0.0051 | 0.0051 | 0.6305 |
| AC109361.1| chr4:106M | -1.3182 | -2.8239 | 0.0047 | 0.0047 | 0.2676 |
| CABLES2| chr20:62M | 0.8582 | 2.791 | 0.0053 | 0.0053 | 2.359  |
| TPGS2| chr18:37M | 1.0585 | 2.759 | 0.0058 | 0.0058 | 2.8821 |
| PLSCR2| chr3:146M | -0.8467 | -2.7271 | 0.0064 | 0.0064 | 0.4288 |
| Gene   | Chromosome | Position | Log2FoldChange | Log10Pval  | Log10FDR  | qValue |
|--------|------------|----------|----------------|------------|-----------|--------|
| AC051619.3| chr15:45M  | -0.5114  | -2.7462        | 0.006      | 0.006     | 0.5997 |
| AP000793.1| chr11:83M  | -0.8667  | -2.7259        | 0.0064     | 0.0064    | 0.4203 |
| DVL3   | chr3:184M  | 1.2316   | 2.8266         | 0.0047     | 0.0047    | 3.4267 |
