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Precision oncology in metastatic colorectal cancer — from biology to medicine

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Abstract | Remarkable progress has been made in the development of biomarker-driven targeted therapies for patients with multiple cancer types, including melanoma, breast and lung tumours, although precision oncology for patients with colorectal cancer (CRC) continues to lag behind. Nonetheless, the availability of patient-derived CRC models coupled with in vitro and in vivo pharmacological and functional analyses over the past decade has finally led to advances in the field. Gene-specific alterations are not the only determinants that can successfully direct the use of targeted therapy. Indeed, successful inhibition of BRAF or KRAS in metastatic CRCs driven by activating mutations in these genes requires combinations of drugs that inhibit the mutant protein while at the same time restraining adaptive resistance via CRC-specific EGFR-mediated feedback loops. The emerging paradigm is, therefore, that the intrinsic biology of CRC cells must be considered alongside the molecular profiles of individual tumours in order to successfully personalize treatment. In this Review, we outline how preclinical studies based on patient-derived models have informed the design of practice-changing clinical trials. The integration of these experiences into a common framework will reshape the future design of biology-informed clinical trials in this field.

The onset and progression of cancer is caused by the accumulation of molecular traits that enable tumour cells to survive, proliferate and elude immunosurveillance, and that foster their adaptability in hostile environments. The identification of tumour molecular maps has guided the design of novel inhibitors that specifically target the altered genes and signalling pathways driving the malignant phenotype. Molecular biomarkers should therefore be used to drive the development of effective targeted therapies as well as to tailor therapy to the individual patient. Yet, applying this logic to colorectal cancer (CRC) has been somewhat daunting given the genetic heterogeneity of these tumours and the paucity of druggable targets[1,2]. Amidst the plethora of molecular alterations identified in CRC over the years, a few basic strategies have emerged for identifying and validating actionable targets (FIG. 1).

With noteworthy exceptions, the 'one gene, one drug' paradigm cannot be universally applied to solid tumours, as originally thought[3]. Even when bona fide oncogenic events are clearly identified, the activity of the corresponding targeted drugs often remains unpredictable owing to intrinsic genetic complexity and a high level of tissue context specificity[4,5]. This complexity is fuelled by multiple putative oncogenic events, occurring concomitantly but independently, that drive tumour evolution along separate pathways. The same pivotal oncogenic pathway, when present in tumour cells of a different lineage, might respond differently to the same drug owing to tissue-specific signalling pathways[6–8].

To overcome these challenges, preclinical models that accurately recapitulate the genomic complexity of cancer have been developed, spanning from molecularly annotated cancer cell lines, which provide the simplest model for pharmacogenomic studies[9,10], to patient-derived models that can enable promising preclinical results to be rapidly translated into clinical trials. Patient-derived organoids (PDOs) provide a powerful model to evaluate cancer cell hierarchies in vitro[11,12] and also to rapidly test personalized treatments[13]. Patient-derived xenograft (PDX) models, in which patient-derived tumour material is transplanted into a mouse, can provide avatars of an individual patient's tumours. In most instances, PDXs allow tumour heterogeneity to be correlated

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Key points

- The efficacy of targeted therapies in patients with solid tumours is largely unpredictable owing to intrinsic genetic complexity and a high level of tissue context specificity.
- The development of patient-derived models that reflect the genetic heterogeneity of colorectal cancer (CRC) constitutes a successful platform for the development of targeted therapies.
- These models have enabled the validation of retrospectively identified biomarkers in clinical trials and the optimization of prospective biomarkers to guide the selection of novel targeted therapies, such as those targeting HER2.
- Longitudinal evaluations of the genomic evolution of CRC enabled by analysis of liquid biopsy samples have further increased the understanding of the mechanisms of resistance to targeted agents.
- Investigations of resistance to targeted therapies have revealed convergence on CRC-specific feedback loops within the MAPK signalling pathway as a core mechanism of survival.
- Co-inhibition with agents targeting EGFR and the specific oncogenic mutation has proved crucial in the clinical development of effective regimens for *BRAF*-mutant CRCs, and has also been demonstrated to be beneficial in the context of $KRAS^{G12C}$-mutant CRC.

with therapeutic responsiveness, thus enabling patterns of cancer dynamics under natural or drug-generated evolution to be identified and the mechanisms of drug resistance to be inferred[14,15]. Alternatively, genetically engineered mouse models provide an in vivo model that can mimic the pathogenesis of both sporadic and inherited CRCs[16–18]. These models might better recapitulate the tumour microenvironment and systemic antitumour immune responses compared with patient-derived models, although their use is not widespread in translational research owing to a limited capacity to reflect invasive disease phenotypes, metastasis and tumour heterogeneity[18].

The analysis of circulating cell-free tumour DNA (ctDNA) obtained from liquid biopsies has been proposed as a method for improving tumour genotyping[19,20]. Liquid biopsy sampling is minimally invasive and can be repeated several times, thus overcoming the spatial and temporal heterogeneity issues associated with tissue biopsy samples[21]. ctDNA analysis was originally used to optimize treatment with anti-EGFR antibodies in the metastatic setting, although most genomic aberrations can now be identified in ctDNA, thus greatly extending the investigative potential of this approach[22]. Analysis of ctDNA has enabled improved identification of resistance mutations[23], and serial monitoring of ctDNA has also been used to assess responses to

therapy[24,25]. Despite the recognition of the analytical validity and clinical utility of this approach[21,26], several hurdles such as cost-effectiveness and the optimization of the pre-analytical steps limit the implementation of ctDNA-based analysis in routine clinical practice and in CRC management guidelines[27].

The development of immunotherapies for the treatment of patients with CRC is not discussed in this Review owing to the different paths that the development of these agents have followed, as reviewed elsewhere[28,29]. We highlight that, despite our understanding of the mechanisms of action and/or resistance to targeted agents being derived from thorough pre-clinical investigation, the mechanisms of activity and resistance to immune-checkpoint inhibitors (ICIs) are still under investigation. Most biomarkers used to guide the use of ICIs in patients with CRC — such as, but not limited to, microsatellite instability high (MSI-H) status, a high tumour mutational burden and pathogenic mutations in *POLE* and *POLD* — are all considered surrogate indicators of increased neoantigen generation by the tumour[28,30–32], with the validation of these features being predominantly clinical[33]. The development of powerful translational models in this field has been limited[34,35], although the PDO–lymphocyte co-culture models developed in the past few years might provide opportunities to develop precision immuno-oncology approaches for the treatment of CRC in the near future[36,37].

In this Review, we describe several experimentally based examples of successful drug development and treatment optimization in patients with metastatic CRC (mCRC), with a prominent focus on targeted agents for which clinical validation was fostered by data from translational studies. An overview of molecular biomarkers that can be used to measure the efficacy and detect the onset of resistance to targeted therapies is also provided. These two aspects are key for selecting the right drug for the right patient, regardless of whether the drug is in development or the post-approval phase, as long as its use is guided by a strong biological rationale.

Development of EGFR-targeted therapies

The HER1 (also known as EGFR) and HER2–HER4 receptor tyrosine kinases are key drivers of tumour cell survival and proliferation[38,39] (FIG. 1). EGFR emerged as a driver of CRC tumorigenesis >30 years ago[40,41], thus paving the way for the clinical development of EGFR-targeted therapies[40,42]. Specifically, two different anti-EGFR antibodies — cetuximab, followed by panitumumab — both showed a statistically significant therapeutic effect, with a 10% improvement in objective response rate (ORR) and an overall survival (OS) advantage of <3 months, relative to placebo, in patients with chemotherapy-refractory metastatic CRC[43,44]. EGFR was used as a predictive biomarker of clinical efficacy in the early trials with cetuximab, and as a result only patients with EGFR-expressing tumours were enrolled, resulting in the initial approval of cetuximab in this setting[44,45]. However, a clear correlation between response to cetuximab and EGFR expression assessed using immunohistochemistry (IHC) was lacking, even in the BOND trial, which was pivotal for the original approval of

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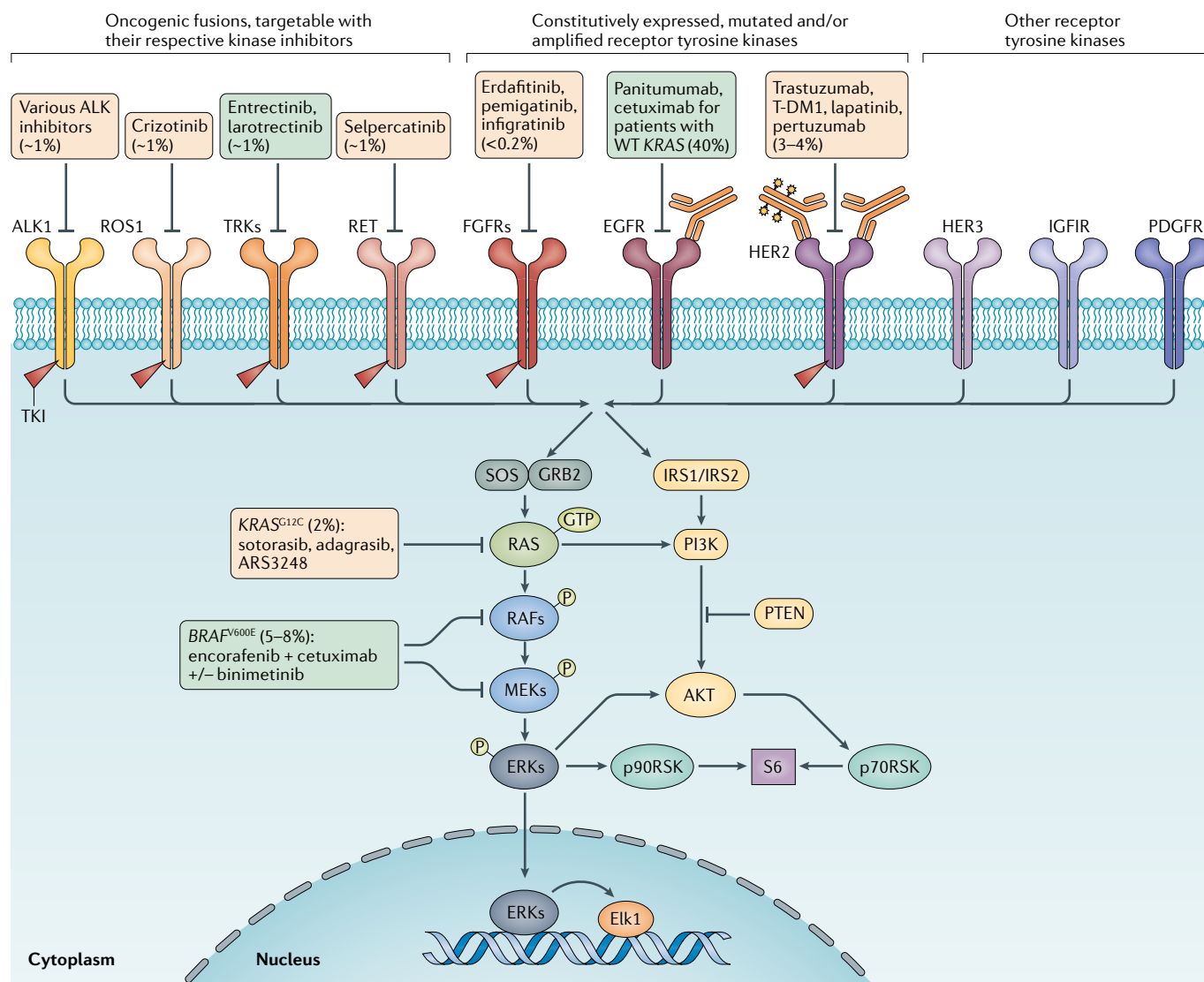[9]These authors contributed equally: Federica Di Nicolantonio, Pietro Paolo Vitiello

Fig. 1 | **Relevant therapeutic targets in metastatic CRC.** The main oncogenic drivers, signalling pathways and their approximate prevalence in patients with metastatic colorectal cancer (CRC). Currently, about 40–45% of CRCs (predominantly those harbouring RAS mutations) lack any targetable alteration. Targeted therapies that are already approved in Europe and/or the USA (green) or have shown efficacy in clinical trials but are currently not approved for patients with CRC (pale orange) are depicted alongside their targets. T-DM1, trastuzumab emtansine; WT, wild-type.

cetuximab[46–48] (TABLE 1). Conversely, panitumumab was initially approved for all patients with metastatic CRC owing to a statistically significant improvement in OS in unselected patients[43,44,46] (TABLE 1).

Primary and acquired resistance. The ultimate clinical development of anti-EGFR antibodies is an emblematic example of a retrospective biomarker assessment strategy (BOX 1; FIG. 2). Soon after the introduction of anti-EGFR antibodies in clinical practice, it became clear that the majority of patients with metastatic CRC fail to respond to these treatments. Subsequent preclinical and clinical studies defined the mechanisms of intrinsic and acquired resistance to these agents, leading to a restriction in their clinical use.

Several studies almost simultaneously revealed that activating mutations in *KRAS*, which occur

predominantly in exon 2 of this gene in 40–45% of patients with metastatic CRC and result in constitutive activation of the MAPK signalling pathway, preclude a response to upstream EGFR blockade[49,50] (FIG. 1). For this reason, retrospective assessments of *KRAS* mutations in samples from patients enrolled in the initial trials testing panitumumab and cetuximab demonstrated a clear advantage of EGFR inhibition only in patients with *KRAS*-wild-type cancers[51–53] (TABLE 1). This lack of benefit from anti-EGFR antibodies in patients with *KRAS*-mutant CRCs was so striking that *KRAS* status was implemented as a predictive biomarker without further prospective evaluation, with the exception of two small phase II trials that demonstrated no statistically significant improvement in disease control with cetuximab monotherapy or cetuximab plus irinotecan in patients with *KRAS*[G13D]-mutant metastatic CRC[54]

Table 1 | **RAS mutations as a negative predictive biomarker of response to anti-EGFR antibodies in patients with metastatic CRC**

Trial	Study characteristics	RAS status	Outcomes[a]
NCI-CO17 (phase II)[47,52]	Cetuximab plus BSC vs BSC for patients with chemorefractory disease (n = 572)	Exon 2 WT (n = 230)	ORR 12.8% vs 0%; mPFS 3.7 months vs 1.9 months (HR 0.40, 95% CI 0.30–0.54; P < 0.001); mOS 9.5 vs 4.8 months (HR 0.55, 95% CI 0.41–0.74; P < 0.001)
		Exon 2 mutant (n = 164)	ORR 1.2% vs 0%; mPFS 1.8 months vs 1.8 months (HR 0.99, 95% CI 0.73–1.35; P = 0.96); mOS 4.5 months vs 4.6 months (HR 0.98, 95% CI 0.70–1.37; P = 0.89)
CRYSTAL (phase III)[259,260]	FOLFIRI plus cetuximab vs FOLFIRI as first-line therapy (n = 1,198)	WT (n = 367)	ORR 66.3% vs 38.6% (OR 3.11, 95% CI 2.03–4.78; P < 0.001); mPFS 11.4 months vs 8.4 months (HR 0.56, 95% CI 0.41–0.76; P < 0.001); mOS 28.4 months vs 20.2 months (HR 0.69, 95% CI 0.54–0.88; P < 0.001)
		Any mutant (n = 460)	ORR 31.7% vs 36.0% (OR 0.86, 95% CI 0.58–1.25; P = 0.4); mPFS 7.4 months vs 7.5 months (HR 1.10, 95% CI 0.85–1.42; P = 0.47); mOS 16.4 vs 17.7 months (HR 1.06, 95% CI 0.86–1.28; P = 0.64)
OPUS (phase II)[261,262]	FOLFOX plus cetuximab vs FOLFOX as first-line therapy (n = 338)	WT (n = 87)	ORR 58% vs 29% (OR 3.33, 95% CI 1.36–8.17; P = 0.0084); mPFS 12 months vs 5.8 months (HR 0.53, 95% CI 0.38–1.09; P = 0.06); mOS 19.8 months vs 17.8 months (HR 0.94, 95% CI 0.56–1.56; P = 0.80)
		Any mutant (n = 167)	ORR 37% vs 51% (OR 0.58, 95% CI 0.31–1.08; P = 0.087); mPFS 5.6 months vs 7.8 months (HR 1.54, 95% CI 1.04–2.29; P = 0.031); mOS 13.5 months vs 17.8 months (HR 1.29, 95% CI 0.91–1.84; P = 0.16)
NORDIC-VII (phase III)[263]	Nordic FLOX vs cetuximab plus FLOX vs cetuximab plus intermittent FLOX as first-line therapy (n = 566)	Exon 2 WT (n = 303)	ORRs 47% vs 46% vs 51%; mPFS 8.7 vs 7.9 vs 7.5 months; mOS 22.0 vs 20.1 vs 21.4 months; all comparisons non-significant
		Exon 2 mutant (n = 195)	ORRs 40% vs 49% vs 42%; mPFS 7.8 vs 9.2 vs 7.2 months; mOS 20.4 vs 21.1 vs 20.5 months; all comparisons non-significant
20020408 (phase III)[43,51,264]	Panitumumab plus BSC vs BSC for patients with chemorefractory disease (n = 463)	WT (including NRAS WT n = 231)	ORR 15% vs 0%; PFS HR 0.38, 95% CI 0.27–0.56
		Any mutant (including NRAS mutations) (n = 196)	ORR 1% vs 0%; PFS HR 0.98, 95% CI 0.73–1.31
PRIME (phase III)[56,59]	FOLFOX plus panitumumab vs FOLFOX as first-line therapy (n = 1,183)	WT (including NRAS) (n = 512)	mPFS 10.1 months vs 7.9 months (HR 0.72, 95% CI 0.58–0.90; P = 0.004); mOS 25.8 vs 20.2 months (HR 0.77, 95% CI 0.64–0.94; P = 0.009)
		Any mutant (including NRAS mutations) (n = 548)	mPFS 7.3 months vs 8.7 months (HR 1.31, 95% CI 1.07–1.60; P = 0.008); mOS 15.5 vs 18.7 months (HR 1.21, 95% CI 1.01–1.45; P = 0.04)
MRC COIN (phase III)[150]	Cetuximab plus FOLFOX or CAPOX vs FOLFOX or CAPOX as first-line therapy (n = 1,630)	Codon 12, 13 and 61 WT (n = 751)	ORR 64% vs 57% (OR 1.35, 95% CI 1.00–1.82; P = 0.049); mPFS 8.6 months vs 8.6 months (HR 0.96, 95% CI 0.82–1.12; P = 0.60); mOS 17.0 months vs 17.9 months (HR 1.04, 95% CI 0.87–1.23; P = 0.67)
		KRAS, NRAS, and BRAF all WT (n = 581)	mOS 19.9 months vs 20.1 months (HR 1.02, 95% CI 0.83 – 1.24; P = 0.86)
		Mutations in KRAS codons 12,13 and 61 (n = 565)	mOS 13.6 months vs 14.8 months (HR 0.98, 95% CI 0.81–1.17; P = 0.80)
		Mutations in BRAF; KRAS and NRAS WT (n = 102)	mOS 7.2 months vs 10.0 months (HR 1.18, 95% CI 0.76–1.81; P = 0.46)
PICCOLO (phase II)[265]	Panitumumab plus irinotecan vs irinotecan as second-line therapy (n = 1,198)	WT (n = 323)	PFS HR 0.68, 95% CI 0.53–0.86; OS HR 0.92, 95% CI 0.73–1.16
		Any mutant (including in KRAS, BRAF, NRAS and PIK3CA) (n = 137)	PFS HR 1.20, 95% CI 0.83–1.74 (P = 0.018); mOS HR 1.64, 95% CI 1.14–2.34 (P = 0.028)
20050181[266,267]	FOLFIRI plus panitumumab vs FOLFIRI as second-line therapy (n = 1,186)	WT (n = 421)	ORR 41% vs 10%; mPFS 6.4 months vs 4.6 months (HR 0.7, 95% CI 0.54–0.91; P = 0.007); mOS 16.2 months vs 13.9 months (HR 0.81, 95% CI 0.63–1.03; P = 0.08)
		RAS mutant (n = 582)	ORR 15% vs 13%; mPFS 4.8 months vs 4.0 months (HR 0.86, 95% CI 0.71–1.05; P = 0.14); mOS 11.8 months vs 11.1 months (HR 0.91 95% CI 0.76–1.10; P = 0.34)
CALGB/ SWOG80405 (phase III)[268,269]	mFOLFOX6 or FOLFIRI plus cetuximab vs mFOLFOX6 or FOLFIRI plus bevacizumab as first-line	WT (n = 429[b])	mPFS 10.9 months vs 11.2 months (HR 0.91, 95% CI 0.73–1.14; P = 0.43); mOS 31.8 months vs 33.6 months (HR 0.9, 95% CI 0.7–1.15; P = 0.38)
		Any RAS mutant (n = 72[b])	mPFS 9.2 vs 11.4 months (HR 0.6, 95% CI 0.34–1.04; P = 0.067); mOS 26.2 vs 22.9 months (HR 1.05, 95% CI 0.6–1.85; P = 0.86)
FIRE-3 (phase III)[270,271]	FOLFIRI plus cetuximab vs FOLFIRI plus bevacizumab as first-line therapy (n = 592)	WT (n = 400)	ORR 65.3% vs 58.7% (OR 1.33, 95% CI 0.88–1.99; P = 0.18); mPFS 10.3 months vs 10.2 months (HR 0.97, 95% CI 0.78–1.20; P = 0.77); mOS 33.1 months vs 25.0 months (HR 0.70 95% CI 0.54–0.9; P = 0.0059)
		Any mutant (n = 188)	ORR 38.1% vs 50.5% (OR 0.6, 95% CI 0.34–1.08); mPFS 7.5 months vs 9.6 months (HR 1.25, 95% CI 0.93–1.68; P = 0.14); mOS 20.2 months vs 20.6 months (HR 1.05, 95% CI 0.77–1.44; P = 0.75)

Table 1 (cont.) | **RAS mutations as a negative predictive biomarker of response to anti-EGFR antibodies in patients with metastatic CRC**

Trial	Study characteristics	*RAS* status	Outcomes[a]
PEAK (phase II)[272,273]	FOLFOX plus panitumumab vs FOLFOX plus bevacizumab as first-line therapy (n = 326)	WT (n = 175)	ORR 65% vs 60% (OR 1.12, 95% CI 0.56–2.22; P = 0.86); mPFS 12.8 months vs 10.1 months (HR 0.68, 95% CI 0.48–0.96; P = 0.15); mOS 36.9 months vs 29.9 months (HR 0.76, 95% CI 0.53–1.11; P = 0.15)
		Non-exon 2 mutations (n = 51)	ORR 58.3% vs 55.6%; mPFS 7.8 months vs 8.9 months HR 1.39 (95% CI 0.73–2.64; P = 0.32); mOS 27.0 months vs 16.6 months (HR 0.41, 95% CI 0.19–0.87; P = 0.02)
CAPRI (phase II)[78,274]	FOLFOX plus cetuximab vs FOLFOX as second-line therapy, following first-line therapy with FOLFIRI plus cetuximab (n = 153)	WT for *KRAS, NRAS, BRAF* and *PIK3CA* (n = 68)	ORR 29.4% vs 9.4%; mPFS 6.9 months vs 5.3 months (HR 0.56. 95% CI 0.33–0.94; P = 0.025); mOS 23.7 months vs 19.8 months (HR 0.57, 95% CI 0.32–1.02; P = 0.056)
		Any mutation in *KRAS, NRAS, BRAF* and *PIK3CA* (n = 51)	mPFS 2.7 months vs 4.4 months (HR 1.7, 95% CI 0.94–3.05; P = 0.07); mOS 11.6 months vs 14.0 months (HR 1.60, 95% CI 0.89–2.96; P = 0.10)

BSC, best supportive care; CAPOX, capecitabine and oxaliplatin; CRC, colorectal cancer; FLOX, folinic acid, 5-fluorouracil (bolus) and oxaliplatin; FOLFIRI, folinic acid, 5-fluorouracil and irinotecan; FOLFOX, folinic acid, 5-fluorouracil (bolus + continuous infusion) and oxaliplatin; mFOLFOX6, modified folinic acid, 5-fluorouracil and oxaliplatin; mPFS, median progression-free survival; mOS, median overall survival; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; WT, wild-type. [a]Outcomes in molecularly specified subgroups are limited to patients with evaluable material. [b]Refers to the primary analysis cohort only.

and a lack of efficacy of the combination of cetuximab and lenalinomide in *KRAS*-mutant metastatic CRC[55]. Furthermore, the design of ongoing as well as subsequent trials involving EGFR-targeted agents was amended to include only patients with *KRAS* exon 2-wild-type disease. Notably, a subgroup analysis of data from the PRIME trial demonstrated that the addition of panitumumab to 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX4) chemotherapy provides favourable OS outcomes compared with FOLFOX4 alone in patients with CRCs harbouring wild-type forms of both *KRAS* and *NRAS*, but not in those with *KRAS*-mutant or *NRAS*-mutant disease[56]. Collectively, these findings prompted the FDA and EMA to revise the approvals for use of cetuximab and panitumumab to include only patients with *KRAS/NRAS*-wild-type CRCs[42,57]. The establishment of *KRAS* and *NRAS* mutations as negative predictive biomarkers of responsiveness to anti-EGFR antibodies in patients with metastatic CRC is a peculiar scenario. Indeed, this was the first clinical setting in medical oncology in which the molecular biomarker is a downstream node (RAS) of the signalling pathway rather than the upstream drug-targeted kinase receptor.

Molecular investigations of negative predictors of response to anti-EGFR antibodies were extended to other transducers or regulators of the MAPK and PI3K–AKT signalling pathways, including alterations in *NRAS, BRAF, PIK3CA* and *PTEN*[57]. Infrequent mutations in exons 3 and 4 of *KRAS* (that occur in 2–5% of patients with metastatic CRC) and in exons 2, 3 and 4 of *NRAS* (in up to 6%) were also included as clinically approved biomarkers because their occurrence, although much less frequent, was strongly correlated with a lack of efficacy in large retrospective analyses[58,59]. However, the findings of several large retrospective studies are discordant regarding the predictive role of aberrations in other downstream components of the EGFR signalling cascade[60–62]. Functional experiments have clearly demonstrated that *BRAF*[V600E] confers resistance to anti-EGFR antibodies in colon cancer cells[60,63–65], and this effect is clinically relevant according to certain retrospective studies[60,66–69]. Unfortunately, none of these studies provides strong confirmation of this effect owing to the small sample sizes analysed, leading to a number of meta-analyses in an attempt to reach an acceptable level of statistical power (reviewed elsewhere[70]). These data confirmed the role of *BRAF* mutations as a predictor of resistance to anti-EGFR antibodies. Nonetheless, the EMA currently recommends pretreatment evaluation of extended *RAS* status (*KRAS* and *NRAS*) to exclude patients with metastatic CRC from receiving cetuximab or panitumumab if they have tumours harbouring mutations in those genes. The FDA label for panitumumab is very similar, while only *KRAS*-wild-type (and not *NRAS*) status is mandatory for treatment with cetuximab. Neither the FDA nor the EMA make any exclusions based on *BRAF* mutation status. Nonetheless, pretreatment *BRAF* testing for the presence of V600E mutations should become the standard of care approach in the management of patients with metastatic CRC, as suggested in the 2016 European Society for Medical Oncology (ESMO) consensus guidelines[71], and the relevance of this alteration is likely to increase in the near future owing to the FDA and EMA approvals of targeted therapies specifically for patients with *BRAF*[V600E]-mutant CRC.

PIK3CA alterations occur in 10–20% of CRCs, and mutations in exon 9 and 20 of this gene have been shown to confer activation of downstream oncogenic signalling that is either dependent on or independent of *RAS* activation, respectively[72,73]. Furthermore, the frequent coexistence of *PIK3CA* mutations or *PTEN* loss in tumours harbouring *RAS* or *BRAF* mutations is probably responsible for the inconclusiveness of their contribution to intrinsic resistance to EGFR-targeted therapies observed in large retrospective analyses; however, at least one meta-analysis has provided evidence of a predictive effect in the context of *KRAS*-wild-type CRC[74–76]. Although not currently incorporated in clinical guidelines[71,77], data from retrospective analyses indicate that an absence of activating mutations in *KRAS, NRAS, BRAF* and *PIK3CA* exon 20 almost doubles the likelihood of response to cetuximab[58]. Furthermore, a persistent absence of these alterations might even confer an advantage in continuing cetuximab in combination with a different chemotherapy backbone beyond first-line therapy, as shown in the phase II CAPRI-GOIM trial[78].

Putative biomarkers of resistance. Several transmembrane receptors have been implicated in intrinsic and/or acquired resistance to EGFR blockade in patients with CRC via parallel oncogenic signalling. For example, both *HER2* (REF.[79]) and *MET*[80,81] amplifications have been characterized in preclinical models and in patients. Overexpression of other receptors, such as AXL[82,83] or EPHA2 (REFS[84,85]), has also been shown to reduce sensitivity to EGFR-targeted therapies in preclinical models, although the clinical effects of these alterations have not been extensively investigated. Rare gene fusions, including those involving *RET*, *ALK*, *ROS1* or *NTRK*[86], are associated with primary resistance to EGFR blockade. Some of these molecular alterations are actionable and either are or might become clinically useful biomarkers. A retrospective analysis using a multigene panel including *HER2* and *MET* amplifications, fusions containing *ALK*, *ROS1*, *NTRK1–3* or *RET*, and *PIK3CA* mutations identified a relevant proportion of patients with metastatic CRC who do not respond to EGFR-targeted therapies, despite having *RAS*/*BRAF*-wild-type disease[87]. The same panel was used in a retrospective analysis of samples from patients who received first-line panitumumab plus chemotherapy in the VALENTINO trial, revealing an enrichment of such mutations in patients with right-sided *RAS*/*BRAF*-wild-type CRCs, which might partially explain the differences in benefit from EGFR-targeted therapies observed in patients with left-sided versus those with right-sided colon cancers[88].

Downstream activation of MAPK signalling is not only the main cause of intrinsic resistance to EGFR blockade in patients with CRC but also the main mechanism of acquired drug resistance[89]. Alterations in *EGFR*, *KRAS*, *NRAS*, *BRAF* or, more rarely, *MEK*[90] emerge during treatment with anti-EGFR antibodies and ultimately cause resistance by reactivating MAPK signalling[91] (FIG. 1). Notably, vertical inhibition of EGFR and MEK is an effective method of impairing tumour growth, both in preclinical models and in patients with acquired alterations in genes encoding MAPK pathway components[92–94]. However, the poor tolerability of this targeted drug combination has hampered its further clinical development.

Several patients with *RAS*-wild-type metastatic CRCs receiving anti-EGFR antibodies develop *RAS* or *EGFR* extracellular domain (ECD) mutations that can also be detected in ctDNA[21,95]. These mutations faithfully reflect preclinical data on the downstream activation of MAPK signalling[90], and fluctuations in the mutant allele frequency (MAF) of a variant can be monitored non-invasively during the course of treatment, both with and without selective therapeutic pressures[96]. Moreover, a direct correlation between the genomic and morphological evolution of CRC metastases monitored using ctDNA and radiological imaging, respectively, has been suggested[97]. Interestingly, the MAFs for specific mutations in ctDNA — such as *KRAS* — follow a 'fluctuating' pattern that reflects the clonal evolution of tumour lesions under the selective pressure of anti-EGFR antibodies. Reductions in the MAFs of *KRAS* and *EGFR* ECD mutations observed in ctDNA during and after treatment with anti-EGFR antibodies have been incorporated into a mathematical model[98,99]. This concept constitutes the biological background for rechallenge therapy with anti-EGFR antibodies after a treatment-free interval, as empirically reported in the CRICKET trial[100]. Several other rechallenge trials, which actively incorporate ctDNA analysis for patient selection, are now ongoing, as described elsewhere[101]. For example, in the CHRONOS study (NCT03227926), liquid biopsy findings from serial blood draws are being used interventionally to triage patients for rechallenge with panitumumab.

Collectively, the available knowledge of mechanisms of resistance enables a 'translational-evidence-based' reclassification of patients with metastatic CRC as either responders — no more than 25–30% of patients — and non-responders to anti-EGFR antibodies[102]. Taken together, this experience provides a pivotal example of how the convergence of clinical experience and preclinical rationale enables the optimization of targeted therapy outcomes, and provides a model for further development in this field.

HER2-targeted therapies

HER2 can trigger the activation of mitogenic and pro-survival signalling pathways in tumours either through homodimerization or heterodimerization with other HER partners[103]. *ERBB2* amplification, leading to receptor overexpression and constitutive kinase activation, has been reported at incidences ranging from

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Box 1 | Prospective or retrospective assessments of biomarkers in CRC?

In the era of targeted therapy, the use of molecular biomarkers to drive the development of targeted drugs and personalize therapy for individual patients is a logical concept. The utility of a specific biomarker, or biomarkers, can be assessed prospectively in clinical trials, retrospectively, or using a combination of these approaches (FIG. 2). To ascertain whether a putative biomarker is suitable for prospective enrichment, at least five points should first be considered:

1. Robust preclinical evidence should be available indicating that the specific gene or signalling pathway of interest is driving tumorigenesis.

2. The experimental drug should have been proven to modulate its putative target.

3. An indication that the experimental drug target is biologically linked with the biomarker of interest should exist, when the target and the biomarker do not overlap.

4. Alterations in the gene or pathway of interest should occur at a low prevalence.

5. A validated assay enabling consistent and accurate quantification of the biomarker in a technically reliable, reproducible and timely manner should be available.

These key points have been applied to the design of successful clinical studies involving patients with *ERBB2*-amplified colorectal cancers (CRCs). In the absence of a strong scientific rationale, patient enrichment is not recommended and 'all comers' should instead be recruited. When studies are conducted in an unselected population, biomarkers can still be assessed retrospectively, even if retrofitting biomarkers to clinical programmes has seldom been a successful strategy. As an example of a rare exception, the identification of *RAS* mutations as a negative predictor of response to anti-EGFR antibodies in patients with metastatic CRC became mainly apparent from retrospective analyses. This strategy was ultimately successful, although the costly phase II–III studies have exposed hundreds of patients to a drug from which they did not benefit. In other scenarios, the combination of retrospective and prospective approaches can be considered, such as in early-phase drug development, in which dose-escalation studies can be performed in unselected patients, while the use of a molecular test can be restricted to an expansion cohort. In the more advanced phases of development, initial equal randomization can be followed by adaptive randomization.
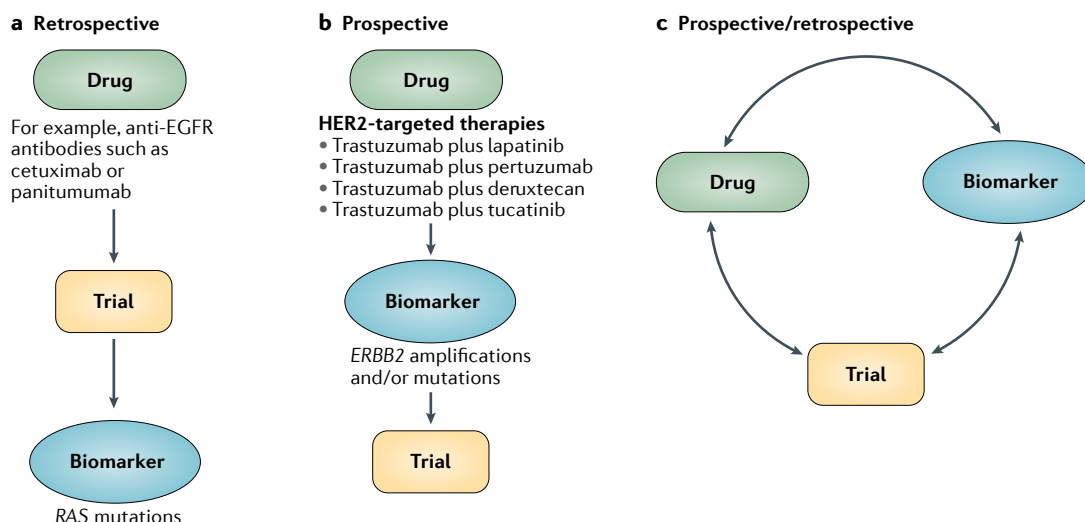
a Retrospective

Drug

For example, anti-EGFR antibodies such as cetuximab or panitumumab

Trial

Biomarker

RAS mutations

b Prospective

Drug

HER2-targeted therapies
- Trastuzumab plus lapatinib
- Trastuzumab plus pertuzumab
- Trastuzumab plus deruxtecan
- Trastuzumab plus tucatinib

Biomarker

ERBB2 amplifications and/or mutations

Trial

c Prospective/retrospective

Drug

Biomarker

Trial

Fig. 2 | **Strategies for the development of biomarker-based targeted therapies in metastatic CRC.** Prospective, retrospective or prospective/retrospective approaches for biomarker-driven drug development are shown. Prospective enrichment is most appropriate whenever the mechanism of action of the agent is intrinsically linked to the biomarker (for example, when the biomarker is the direct target of the drug). Retrospective approaches enable the identification of biomarkers that drive the development of targeted agents associated with clinical responses in unselected patients. The combination of both of these approaches enables the optimal candidate population for a specific treatment to be identified after initial prospective enrichment. The use of molecular biomarkers to drive the development of targeted drugs is discussed in BOX 1.

1.8% to 22% across different cohorts[104] with differences probably reflecting the application of more-relaxed or more-stringent criteria for distinguishing polysomy of chromosome 17 (at which the *ERBB2* locus resides), copy number gains and focal high-grade gene amplifications. Taken together, data from most retrospective analyses demonstrate that the prevalence of *ERBB2* amplification is consistently low (<5%) when diagnostic criteria are properly harmonized and implemented[105,106]. Several reports highlight an enrichment for *KRAS*-wild-type alleles in patients with *ERBB2*-amplified CRCs[107–109].

Data from certain studies suggest that distal carcinomas located in the rectum and left colon are more likely to harbour *ERBB2* amplification than proximal carcinomas of the caecum and right colon[109–111]; however, other analyses have failed to confirm these observations[112–114]. Initial evidence suggests that HER2-positive tumours grow more aggressively, given the increased levels of HER2 expression seen in patients with advanced-stage cancers and in those with a higher metastatic burden[115,116]. In a study involving 1,645 patients with CRC of all stages (I–IV), a trend towards worse OS emerged in those with HER2-positive disease compared with those with HER2-negative tumours[110]. Similarly, in the PETACC-8 study, the 66 patients with stage III HER2-positive colon cancers (out of 1,689 evaluated) had shorter time to disease recurrence and inferior OS[112]. In general, evaluation of the negative prognostic effects of *ERBB2* amplification are complicated by the limited prevalence of such alterations in patients with CRC and the lack of standard criteria for setting HER2-positivity thresholds.

The Cancer Genome Atlas project identified *ERBB2* mutations in 9 of 212 CRC samples examined (4%), with three of these samples concomitantly harbouring *ERBB2* amplifications[1]. Similarly, an independent

sequencing study of 69 CRC samples pinpointed three *ERBB2*-mutated cancers[117]. Many of these mutations are identical to those found in patients with breast cancer, including the kinase-domain mutations V842I, V777L and L755S, and the ECD mutation S310F, and have been experimentally demonstrated to drive constitutive HER2 signalling and to induce a tumorigenic phenotype in CRC cell lines[118]. Notably, in the metastatic setting, patients with *ERBB2*-mutant tumours seem to have worse OS than patients with *ERBB2*-wild-type tumours[119], suggesting that this alteration is prognostically relevant.

HER2 and resistance to EGFR inhibition. An initial clue regarding the role of *ERBB2* amplification as a negative predictor of response to anti-EGFR antibodies came from the preclinical observation that CRC xenografts derived from patients with metastatic disease and wild-type forms of *KRAS*, *NRAS* and *BRAF* who were nonetheless refractory to EGFR blockade were enriched for HER2 overexpression, owing to high-grade *ERBB2* amplifications[107]. This observation is supported by exogenous HER2 overexpression in cetuximab-sensitive CRC cell lines, which causes resistance to this antibody, thereby functionally validating the suggestion that HER2 hyperactivation removes the need for EGFR signalling[120].

Several retrospective case series have documented an association between HER2-positivity and worse outcomes in response to anti-EGFR antibodies. In the first study, median progression-free survival (PFS) and OS durations were reduced by almost 50% in patients with *ERBB2*-amplified tumours (*n* = 13) relative to those with non-amplified tumours (*n* = 220) who received cetuximab either alone or in combination with chemotherapy[79]. In a second cohort of 162 patients who

received cetuximab or panitumumab, with or without chemotherapy, six patients (3.7%) with *ERBB2* amplifications detected in all neoplastic cells had a substantially shorter PFS and OS[121]. In another cohort, patients who received first-line cetuximab or panitumumab plus folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) had a lower ORR with a trend towards worse survival outcomes, compared with a comparator group of patients with HER2-negative disease[116].

ERBB2-activating mutations have also been shown to confer resistance to cetuximab and panitumumab in CRC cell lines[118]. Furthermore, similar to that observed for *ERBB2* amplifications, *ERBB2*-mutant PDX models from patients with metastatic CRC have been proven to be refractory to EGFR inhibition[118]. The clinical implications of these observations, and whether or not patients with *ERBB2*-mutant metastatic CRCs are poorly responsive to anti-EGFR antibodies, and should therefore be excluded from receiving such treatments, remain to be established owing to an absence of data from prospective trials.

Targeting HER2 alterations. *HER2* amplifications can be targeted using monoclonal antibodies (such as trastuzumab or pertuzumab) or tyrosine-kinase inhibitors (TKIs; such as lapatinib or tucatinib). Notably, trastuzumab added to standard chemotherapy in patients with HER2-positive breast cancer was the first example of an effective targeted therapy for patients with solid tumours[122]. Early trials in which patients with metastatic CRC received trastuzumab plus chemotherapy, however, were inconclusive owing to suboptimal HER2 testing and an inadequate sample size[123,124], which dampened interest in HER2 as an actionable oncogene in this disease for a period of time. A renewed interest in the small (3–5%) but clinically significant subpopulation of patients with HER2-positive CRC was spurred by results from proof-of-concept preclinical experiments in PDX models of *ERBB2*-amplified metastatic CRC, which showed that dual blockade of the HER signalling pathway with trastuzumab and the dual EGFR–HER2 TKI lapatinib is required for rapid and long-lasting tumour regression[120]. Mechanistically, the synergistic activity of this combination is ascribed to the ability of trastuzumab to prevent HER3 phosphorylation, which occurs during protracted treatment with lapatinib owing to compensatory transcriptional upregulation of HER3 (REF.[120]). Preclinical observations rapidly prompted the design of the phase II HERACLES-A trial, in which the combination of trastuzumab plus lapatinib was tested in patients with HER2-positive metastatic CRC[125–127]. A total of 914 patients with chemotherapy-refractory *KRAS*-wild-type metastatic CRC were screened, and 46 (5%) were found to have HER2-positive tumours. The ORR of the 27 patients eligible for inclusion in the trial was 30%, including one complete response that led to >5 years without evidence of disease. Overall, disease control was achieved in 74% of patients, with a median duration of response, PFS and OS of 9.5, 5.2 and 11.5 months, respectively. This chemotherapy-free regimen was well tolerated; most patients had grade 1 or 2 adverse events only. Interestingly, all eight

responders had an *ERBB2* copy number >9.45. An analogous correlation between best objective response and level of *ERBB2* amplification was found using ctDNA to detect *ERBB2* copy number[22]. The observation that the magnitude of *ERBB2* amplification is associated with response can probably be explained by the higher dependency on HER2 oncogenic signalling of tumours featuring abnormally high *ERBB2* gene dosages[125]. Following HERACLES-A, the optimization of HER2-targeted therapies for patients with CRC diversified into two branches, involving either monoclonal antibodies or TKIs (TABLE 2).

In the second HERACLES trial (HERACLES-B)[128], patients with HER2-positive metastatic CRC received pertuzumab, a monoclonal antibody that inhibits ligand-induced HER2–HER3 heterodimerization[129], plus trastuzumab emtansine (T-DM1), an antibody–drug conjugate linking trastuzumab to the tubulin-binding agent DM1 (REF.[130]). Despite a disease control rate (DCR) of 80% and a median PFS duration of 4.8 months, HERACLES-B failed to meet the primary end point (ORR ≥30%), with an ORR of 10%. The suboptimal ORR probably relates to the lower dose of trastuzumab delivered by T-DM1 and to the fact that CRCs are typically poorly sensitive to microtubule-disrupting agents. Another trastuzumab immunoconjugate with DM1 replaced by the topoisomerase 1 inhibitor deruxtecan (trastuzumab deruxtecan (T-DXd)) has since been developed, and shows a better antibody-to-payload ratio than T-DM1 (7.7 versus 3.5, respectively)[131,132]. Indeed, DESTINY-CRC01, a trial exploring the safety and efficacy of T-DXd, showed more impressive levels of activity in 53 patients with HER2 IHC staining score of 3+ or 2+ and in situ hybridization-positive metastatic CRC[133]. The confirmed ORR in this trial was 45.3% (one complete response and 23 partial responses) with a DCR of 83%[133]. Notably, these results were achieved in patients who had failed to respond to and/or had disease progression on both EGFR-targeted and HER2-targeted therapies.

A combination of full-dose trastuzumab and pertuzumab explored in the MyPathway trial was also found to be active in 57 patients with treatment-refractory HER2-positive mCRC[134], with results consistent with those of HERACLES-A. Objective responses were observed in 18 patients (32%) and clinical benefit (defined as disease control lasting ≥4 months) was observed in 25 (44%); median PFS was 2.9 months, and median OS was 11.5 months. A similar ORR (34%) was also documented in an interim response evaluation from a second trial involving this regimen, the ongoing TRIUMPH study[135], in which patients with *ERBB2*-amplified mCRCs were identified by tumour tissue and/or ctDNA analyses and selectively enrolled.

A second strategy leveraging the PDX-based methodological pipeline[136] that led to the initiation of the HERACLES studies pursued the clinical development of new selective HER2 TKIs, such as tucatinib and neratinib. In the MOUNTAINEER trial, tucatinib was tested in combination with trastuzumab in 22 patients with pretreated *KRAS*/*NRAS*-wild-type, *ERBB2*-amplified and/or HER2-overexpressing metastatic CRCs[137]. Data from this trial demonstrated an ORR of 55%

Table 2 | **Biomarker-selected HER2-targeted therapies in patients with metastatic CRC**

Trial	Biomarker	Description	Outcomes	Grade ≥3 AEs (% of patients)
HERACLES-A (phase II)[22,125,127]	HER2 positivity (HERACLES pathological criteria[a])	Trastuzumab plus lapatinib in patients with chemorefractory KRAS-WT disease (n = 27)	ORR 28%; mPFS 4.7 months in patients with ERBB2 GCN >9.5 and 3.7 months in patients with ERBB2 GCN <9.5; mOS 10.0 months	Fatigue 15%, rash 4%
My Pathway (phase II)[134]	HER2 positivity assigned based on IHC (3+ staining), FISH (ERBB2:CEP17 >2.0) and/or NGS (ERBB2 copy number gain)	Trastuzumab plus pertuzumab in patients with KRAS-unselected chemorefractory disease (n = 56)	All patients (n = 56): ORR 32%; mPFS 2.9 months; mOS 11.5 months KRAS-WT (n = 43): ORR 40%; mPFS 5.3 months; mOS 14.0 months	Gastrointestinal 8%, left ventricular dysfunction 2%
TRIUMPH (phase II)[135]	ERBB2 amplifications determined using tissue and/or ctDNA analysis	Trastuzumab plus pertuzumab in patients with chemorefractory RAS-WT disease (n = 18)	ORR 35% (tissue-positive), 33% (ctDNA-positive); mPFS 4 months	Cardiac toxicities 10.5%
MOUNTAINEER (phase II)[137]	HER2 positivity determined using IHC (3+ or 2+ staining and FISH-positive), FISH and/or NGS	Trastuzumab plus tucatinib in patients with chemorefractory RAS-WT disease (n = 26)	ORR 55%; mPFS 6.2 months; mOS 17.3 months	Diarrhoea 4%
HERACLES-B (phase II)[128]	HER2 positivity (HERACLES pathological criteria[a])	T-DM1 plus pertuzumab in patients with chemorefractory RAS-/BRAF-WT disease (n = 31)	ORR 10%, DCR 80%, mPFS 4.8 months	Thrombocytopenia 6.5%
DESTINY-CRC01 (phase II)[133]	HER2 positivity Cohort A: HER2 IHC 3+ or IHC 2+ staining and ISH-positive (n = 53) Cohort B: IHC 2+ staining and ISH-negative (n = 7) Cohort C: IHC 1+ staining (n = 18)	T-DXd in patients with disease progression on two or more prior regimens (n = 78)	Cohort A: ORR 45.3% (43.8% in patients who had previously received HER2-targeted therapy); DCR 83%; mPFS and mOS not reached No responses observed in cohorts B and C	Thrombocytopenia 48.7%, fatigue 10%, nausea 2%, interstitial lung disease 3.9% (including two treatment-related deaths)

AEs, adverse events; CRC, colorectal cancer; ctDNA, circulating tumour DNA; DCR, disease control rate; FISH, fluorescence in situ hybridization; GCN, gene copy number; IHC, immunohistochemistry; ISH, in situ hybridization; mOS, median overall survival; mPFS, median progression-free survival; NGS, next-generation sequencing; ORR, objective response rate; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; WT, wild-type. [a]Defined as a HER2 IHC score of 3+ in ≥50% of cells or a HER2 IHC score of 2+ and an ERBB2 to CEP17 ratio of >2 in ≥50% of cells by FISH[105].

(12 partial responses) with median PFS and OS durations of 6.2 months and 17.3 months, respectively.

The SUMMIT multi-histology basket trial tested the safety and efficacy of neratinib monotherapy in patients with breast cancer or CRC harbouring mutations in ERBB2 or ERBB3. The ORR was 32% in patients with breast cancer, although none of the 12 patients with CRC responded[138]. These negative outcomes are in line with preclinical results from PDX models of CRC, in which tumour regression is observed only when neratinib is combined with trastuzumab[118]. This lack of response to neratinib monotherapy could reflect, among other things, the frequent occurrence of co-existing KRAS and PIK3CA mutations in patients with ERBB2-mutant CRC[119].

The ORRs in selected cohorts of patients receiving these different HER2-targeted therapy regimens is consistently around 30%, which compares favourably with the ORRs typically achieved with other approved third-line therapies, such as the multikinase inhibitor regorafenib (ORR 1–4%)[139,140] and trifluridine plus tipiracil chemotherapy (ORR 2%)[141,142]. The results with HER2-targeted therapies are particularly meaningful considering that all trials enrolled heavily pretreated patients, who had often already received both of these other treatments.

Finally, owing to no reasonable expectation of benefit from anti-EGFR antibodies in patients with ERBB2-amplified metastatic CRC[116], we advocate the routine assessment of ERBB2 amplification in the molecular diagnostic work-up to spare patients potential toxicities associated with EGFR-targeted therapy. Such patients should instead be referred to investigational treatment with HER2-targeting agents.

Resistance to HER2-targeted therapies. Next-generation sequencing (NGS) of ctDNA obtained from 29 patients enrolled in the HERACLES trials (A and B) was conducted using a targeted panel in an attempt to uncover the molecular determinants of resistance to HER2-targeted therapies[97]. Alterations in RAS and/or RAF genes were detected at baseline in six of seven patients (86%) with treatment-refractory disease but only in 3 of 22 (14%) who derived clinical benefit. These alterations had a high MAF, suggesting a clonal origin as dominant 'trunk' mutations. Among patients who had disease control, low-MAF (subclonal) KRAS mutations and BRAF amplifications were identified at disease progression, together with alterations in HER2, EGFR, PIK3CA and PTEN. In one patient who had a mixed radiological response — with some metastatic lesions enlarging and others shrinking over the course of treatment — the rapid processing of post-mortem tissue samples from the different metastases revealed reduced or absent HER2 expression in progressing lesions, with one lesion also having heterogeneous EGFR amplification[97]. An exploratory analysis of tumour samples from patients who participated in the MyPathway study confirmed

these observations[134]. The ORR was only 8% in the subgroup of patients with tumours harbouring *KRAS* mutations (23%) versus 40% in those with *KRAS*-wild-type tumours. Likewise, the ORR was lower in patients with *PIK3CA* mutations (13%) than in patients with *PIK3CA*-wild-type tumours (43%). Overall, molecular data from the HERACLES and MyPathway trials highlight the relevance of the RAS–MAPK and PI3K–AKT signalling pathways in mediating both de novo and acquired resistance to HER2-targeted therapies.

BRAF-targeted therapies

BRAF mutations are found in about 10–15% of all CRCs[60,143–145], and approximately 90% of them involve a single amino acid substitution of valine by glutamate within codon 600 (V600E)[146]. This mutation enables RAS-independent constitutive activation of the MAPK signalling pathway and is generally mutually exclusive with *KRAS* and *NRAS* mutations, indicating that a single alteration in the MAPK pathway is sufficient to enable tumorigenic activity[143,147–151]. *BRAF*-mutant CRCs frequently have a CpG island methylator phenotype and are often also MSI-H (around 50% of all operable MSI-H CRCs contain *BRAF* mutations[151], although this frequency decreases to 11% in the metastatic setting[128]).

The occurrence of *BRAF* mutations defines a specific disease subtype with a unique patient population, and an unfavourable prognosis in the metastatic setting. Generally, *BRAF*-mutant tumours arise in the right-sided proximal colon, are more prevalent in women and in older patients (>65 years old), have a mucinous and poorly differentiated histology[146], and spread preferentially to the peritoneum or distant lymph nodes[152], making these patients less likely to be eligible for metastasectomy. Generally, these patients have shorter OS durations (median 10.4 months versus 34.7 months in patients with *BRAF*-mutant versus *RAS/BRAF*-wild-type stage IV CRC)[146,152,153], and shorter recurrence-free survival durations at earlier disease stages[154,155], although this effect is seen mainly in patients with microsatellite-stable *BRAF*-mutant tumours[144,156,157]. This observation is related to the fact that MSI-H tumours are generally associated with a favourable prognosis[144,151], although some data suggest that the prognostic implications of *BRAF* mutations remain relevant in MSI-H cancers[154,158,159]. The shorter median OS duration associated with *BRAF* mutations is likely to be solely attributed to the prognostic effect of the mutations[153,160] and not changes in sensitivity to standard-of-care chemotherapies, such as oxaliplatin and irinotecan. Results from a large-cohort, retrospective analysis of NGS data from patients with *BRAF*-mutant CRC revealed that the poor prognostic association of such mutations is limited to *BRAF*^V600E (REF.[161]). Indeed, most non-V600E mutations in *BRAF*, which occurred in 2.2% of all patients tested, conferred an excellent prognosis with improved OS, consistent with earlier reports[161,162].

Collectively, these data suggest that *BRAF* mutations are a major driver of right-sided tumours, given the strong association between *BRAF* mutations and proximal CRCs. Such alterations might contribute to the differences in prognosis and metastatic spread in patients with tumours harbouring these alterations.

Targeting BRAF-mutant CRCs. Small-molecule BRAF kinase inhibitors, such as vemurafenib and dabrafenib, induce dramatic ORRs of 50–80% in patients with *BRAF*^V600E-mutant metastatic melanoma, and are approved in this setting[163,164]. However, only 5% of patients with *BRAF*^V600E-mutant metastatic CRCs respond to vemurafenib[165]. Encorafenib, another potent and selective BRAF kinase inhibitor, also failed to show any activity as a monotherapy in a similar cohort[166]. All cohorts included patients with melanomas or CRCs harbouring the same mutation (*BRAF*^V600E); therefore, the unexpectedly negative results in patients with CRC were both clinically disappointing and biologically puzzling. Indeed, these data fundamentally challenged the founding principle of targeted therapy. How this molecular mystery was tackled provides a good example of an excellent back-and-forth research effort between the laboratory and clinical settings. In brief, analysis of biopsy samples obtained from patients with *BRAF*^V600E-mutant melanoma revealed that suppression of the MAPK signalling pathway is necessary for a response to therapy[167]. However, investigations of CRC cells harbouring the same mutation revealed only transient suppression of MAPK signalling and rapid re-accumulation of phosphorylated ERK (pERK) within 6 hours of exposure to vemurafenib[7]. Transient and incomplete inhibition of MAPK signalling therefore became the putative mediator of resistance to BRAF inhibitors in CRC. Indeed, the feedback reactivation of MAPK signalling under BRAF or MEK inhibition seems to be driven by EGFR-mediated activation of RAS and CRAF phosphorylation[6,7]. This molecular feedback can be explained by BRAF inhibition conferring a reduction in MEK and ERK kinase activity, which in turn leads to reduced activation of CDC25 phosphatases and ultimately triggers an increase in EGFR phosphorylation (pEGFR) owing to decreased dephosphorylation[6].

Melanomas originate from the neural crest and therefore do not express EGFR, making this feedback loop ineffective and rendering these cancers sensitive to BRAF inhibitors. However, CRCs originate from epithelial cells in which EGFR is generally constitutively expressed[6,7]. Interestingly, both in vitro and in vivo experiments have confirmed that anti-EGFR agents do indeed synergize with BRAF inhibitors in the context of CRC[6,7]. Particularly, exposing cell lines to BRAF and EGFR inhibitors resulted in the abrogation of AKT, MEK and ERK phosphorylation.

Targeting BRAF, EGFR and MEK. Elucidation of the central role of EGFR in primary resistance to BRAF inhibitors led to four trials evaluating combinations of different BRAF inhibitors (vemurafenib, dabrafenib and encorafenib) and anti-EGFR antibodies (cetuximab and panitumumab) in patients with *BRAF*^V600E-mutant CRCs[168–170]. BRAF inhibition can induce EGFR upregulation; therefore, adding a MEK inhibitor to the combination of a BRAF and an EGFR inhibitor might enable more effective MAPK inhibition.

This reasoning was supported by a pharmacodynamic analysis of paired pretreatment and on-treatment biopsy samples from patients receiving inhibitors of either two kinases (BRAF and EGFR or BRAF and MEK) or three kinases (BRAF, EGFR and MEK), resulting in mean on-treatment decreases in pERK of 37%, 41% and 60% with the two doublet regimens and the triplet regimen, respectively[171]. The latter finding is in line with the 76% decrease observed in patients with *BRAF*-mutant melanoma receiving dabrafenib alone[172]. Notably, another trial evaluating the combination of the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib revealed limited efficacy, with an ORR of 12%, despite this combination inducing sustained MAPK suppression in *BRAF*[V600E]-mutant CRC cell lines[173,174].

Following these observations, several phase I–II trials involving different triplet regimens in second-line or later-line settings were conducted[170,171]. The first triplet regimen to move forward to a phase III trial was the combination of encorafenib, the MEK inhibitor binimetinib and cetuximab in the BEACON trial, which demonstrated ORRs of 26%, 20% and 2% in the triplet, doublet (cetuximab plus encorafenib) and control (cetuximab plus irinotecan-based chemotherapy) arms, respectively. Median PFS durations were 4.3, 4.2 and 1.5 months and median OS durations were 9, 8.4 and 5.4 months across the three arms, respectively[175]. Interestingly, this large-cohort study failed to reveal a clinically relevant increase in survival outcomes with the addition of binimetinib to cetuximab plus encorafenib. On the basis of these results, both the FDA and EMA approved encorafenib plus cetuximab for patients with previously treated *BRAF*[V600E]-mutant metastatic CRCs. More recently, data from stage 1 of the phase II ANCHOR study, including 41 patients receiving the same triplet combination as a first-line therapy, has shown encouraging results compared with those of the BEACON study, with an ORR of 50%, a DCR of 85%, a median PFS duration of 4.9 months and immature OS at the latest data cut-off[176]. Details of the trials discussed above involving BRAF-targeted therapies are presented in TABLE 3.

Targeting BRAF, EGFR and PI3K. Activation of the PI3K signalling pathway has also been hypothesized to mediate resistance to BRAF inhibitors[177]. A triplet combination of cetuximab plus encorafenib and the PI3Kα-specific inhibitor alpelisib produced an ORR of 18% and a median PFS duration of 4.2 months with a DCR of 93%[178]. However, patients in the triplet arm had a higher incidence of toxicities than those in the cetuximab–encorafenib doublet arm (grade 3–4 adverse events in 79% versus 69% of patients, respectively), without a significant improvement in the extent of clinical benefit (median PFS in the doublet group was 3.7 months). Notably, alpelisib is ineffective in the presence of molecular alterations leading to loss of PTEN function[179]; these alterations are present in nearly 40% of patients with *BRAF*[V600E]-mutant CRC[1].

Resistance to BRAF-targeted therapies. Despite an initial response, patients with CRC inevitably have disease relapse after a few months of treatment with BRAF inhibitor combination regimens and several mechanisms of acquired drug resistance have been described[165,178,180–185]. Amplification or mutation of *KRAS* or *NRAS* is a common mechanism by which

Table 3 | **Trials involving BRAF-targeted therapies for patients with *BRAF*[V600E]-mutant mCRC**

Trial	Description	Outcomes	Grade ≥3 AEs (% of patients)
NCT00405587 (phase II)[165]	Dose-expansion study exploring the efficacy of vemurafenib (n = 21)	ORR 5%, mPFS 2.1 months, mOS 7.7 months	SCC of the skin 23.8%, fatigue 4.8%, diarrhoea 4.8%
SWOG1406 (phase II)[275]	Irinotecan plus cetuximab plus vemurafenib vs irinotecan plus cetuximab in patients with disease progression on one or two prior regimens (n = 106)	ORR 17% vs 4% (P = 0.05), DCR 67% vs 21% (P < 0.001), mPFS 4.4 months vs 2.0 months (HR 0.50, 95% CI 0.26–0.66; P < 0.001)	Neutropenia 28% vs 7%, anaemia 13% vs 0%, nausea 15% vs 0%
NCT01791309 (phase Ib)[169]	Panitumumab plus vemurafenib in patients with disease progression on one or more prior regimens (n = 15)	ORR 17%, mPFS 3.2 months, mOS 7.6 months	Fatigue 7%, neutropenia 7%
NCT01750918 (phase I–II)[171]	Panitumumab plus dabrafenib vs panitumumab plus trametinib vs panitumumab plus dabrafenib plus trametinib in patients who received no or up to four prior lines of therapy (n = 142)	ORR 10% vs 0% vs 21%; mPFS 3.5 months (95% CI 2.8–5.8) months vs 2.6 months (95% CI, 1.4–2.8) vs 4.2 months (95% CI, 4.0–5.6); mOS 13.2 months (95% CI, 6.7–22.0) vs 8.2 months (95% CI, 6.5–9.4) vs 9.1 months (95% CI, 7.6–20.0)	Overall 45% vs 67% vs 70%, fatigue 0% vs 0% vs 7%, rash 0% vs 6% vs 11%, pyrexia 0% vs 0% vs 4%, dermatitis acneiform 0% vs 18% vs 10%
BEACON (phase III)[175]	Cetuximab plus encorafenib plus binimetinib vs cetuximab plus encorafenib vs cetuximab plus FOLFIRI/irinotecan in patients with disease progression on one or two prior regimens (n = 665)	ORR 26% vs 20% vs 2%; mOS 9 months (HR for death vs cetuximab plus chemotherapy 0.52, 95% CI 0.39–0.70; P < 0.001) vs 8.4 months (HR for death vs cetuximab plus chemotherapy 0.60, 95% CI 0.45–0.79; P < 0.001) vs 5.4 months	Overall 58% vs 60% vs 61%, diarrhoea 10% vs 2% vs 10%, dermatitis acneiform 2% vs 1% vs 3%
NCT01719380 (phase Ib)[178]	Cetuximab plus encorafenib vs cetuximab plus encorafenib plus alpelisib in patients who had received one to four prior lines of therapy (n = 54)	ORR 19.2% vs 17.9%, mPFS 3.7 months vs 4.2 months	Fatigue 11.5% vs 3.6%, diarrhoea 3.8% vs 3.6%, dermatitis acneiform 0% vs 3.6%, pyrexia 0% vs 3.6%
ANCHOR–safety lead-in (phase II)[176]	Cetuximab plus encorafenib plus binimetinib as first-line therapy (n = 40)	ORR 50%, DCR 85%, mPFS 4.9 months	NA

AEs, adverse events; DCR, disease control rate; FOLFIRI, folinic acid, 5-fluorouracil and irinotecan; mCRC, metastatic colorectal cancer; mPFS, median progression-free survival; mOS, median overall survival; NA, not available; ORR, objective response rate; SCC, squamous cell carcinoma.

$BRAF^{V600E}$-mutant CRCs overcome the effects of targeted inhibition[171,181,182,184]. An analysis of CRC tumour material using Sanger sequencing revealed that *KRAS* and *BRAF* mutations are generally mutually exclusive[186–188]. Thus, concomitant oncogenic activation of KRAS and BRAF signalling is postulated to result in activation of cell-cycle inhibitory proteins, leading to oncogenic stress, senescence and subsequent counter-selection during tumour progression[189]. Nevertheless, the use of more-sensitive techniques, such as droplet digital PCR, indicates that clones harbouring mutations in both *KRAS* and *BRAF* can be detected in CRC samples, albeit at low MAFs[165]. Accordingly, monitoring *KRAS* status is advisable in patients with *BRAF*-mutant CRC who are receiving targeted therapies. *KRAS* alterations that emerge during treatment with BRAF inhibitors can not only induce resistance, but might also cause paradoxical upregulation of MAPK signalling, mediated by RAF dimerization and CRAF activation, leading to the promotion of tumour growth[190,191].

Patients receiving combinations of targeted therapies can have selective *BRAF* amplification together with acquired mutations in *MEK1* (REFS[181,182,184]). Amplification of *MET* has also been reported as a mechanism of secondary resistance in patients with *BRAF*-mutant CRC[183,185]. Overall, similar to the experience with anti-EGFR antibodies in patients with *BRAF*-wild-type and *RAS*-wild-type CRCs, *BRAF*-mutant CRCs evade targeted inhibition through the emergence of molecular alterations that reactivate MAPK signalling.

NTRK and other gene fusions

The development of effective targeted therapies for oncogenic gene fusions has become clinically feasible only in the past two decades, owing to advances in molecular diagnostic techniques[192]. In a subset of CRCs and most other epithelial cancers, gene fusions including those involving *NTRK*, *ROS*, *ALK* and *RET* are key oncogenic drivers, albeit only in a small minority of patients (<2.5%)[86,193]. Nonetheless, such fusions are clinically interesting because they are all now pharmacologically actionable with the potential to confer better clinical outcomes than those achieved with standard-of-care CRC therapies.

NTRK fusions were originally detected in a CRC specimen[194], although these fusions are detectable in <0.5% of all CRCs[195–198]. The first TRK inhibitors to enter clinical development are entrectinib (which also inhibits *ALK* and *ROS1*) and larotrectinib[199,200]. Both drugs were tested in multiple phase I/II basket trials involving both paediatric and adult patients with advanced-stage solid tumours[201,202] with results so impressive (ORRs of 79% and 57%, respectively) that they led the FDA (and the EMA for larotrectinib) to grant tumour-agnostic approval for patients with solid tumours harbouring *NTRK* fusions[203,204]. Subgroup analyses of data from patients with gastrointestinal cancers receiving either larotrectinib or entrectinib showed a lower response rate than in the global population, although CRC-specific statistical analyses cannot be performed owing to the very low number of patients (four for each pooled analysis)[205,206].

Gene fusions involving *ALK* or *ROS1* have also been identified and characterized in 0.2–2.4% of patients with CRC, with a similar pattern of distribution to that of *BRAF* mutations, including associations with female sex, older age (>65 years) and co-occurrence with MSI status[86]. Owing to the higher prevalence of *ALK*-containing or *ROS1*-containing fusions in patients with non-small-cell lung cancer (NSCLC), half a dozen ALK and/or ROS1 inhibitors are available[207], albeit none are currently approved specifically for the treatment of patients with CRCs harbouring the same translocation. Interestingly, entrectinib was shown to be active in a patient with *ALK* translocation-positive CRC[208], and at least two ongoing clinical trials are enrolling patients with CRCs harbouring *ALK* alterations (NCT03792568 and NCT02568267).

RET fusions are also a rare occurrence in patients with CRC (0.2% of all cases) and are predominantly detected in older patients with right-sided cancers and are associated with a poor prognosis compared with *RET*-negative cancers[209]. Nonetheless, a patient with metastatic CRC harbouring a *RET* fusion had a complete response to the experimental RET inhibitor agerafenib, and at the time of reporting remained disease-free after 19 months of treatment[209]. *FGFR* rearrangements are a similarly rare occurrence in patients with metastatic CRC (<0.2% of all cases), albeit several treatments are either already available or in advanced clinical testing such as erdafitinib, pemigatinib, and infigratinib[210,211].

Resistance to NTRK inhibitors. As with the other targeted agents, the onset of resistance is the main limitation to the clinical efficacy of NTRK inhibitors. Mutations that dramatically decrease the binding affinity of the drug to the kinase domain of the fusion protein are the most prevalent mechanism of resistance to larotrectinib[212–214]. Interestingly, the mutations mediating resistance to NTRK inhibitors are paralogous to those that mediate resistance to ALK and ROS1 inhibitors[215]. Analysis of liquid biopsy samples enabled the early identification of putative mutations associated with resistance to NTRK inhibitors that were promptly modelled using PDXs, thus paving the way for the accelerated testing of second-generation inhibitors that can overcome the effects of these mutations, such as selitrectinib and repotrectinib that were developed alongside the first-generation drugs[213,216]. More recently, resistance to NTRK inhibitors has been linked to off-target genomic alterations affecting KRAS, BRAF and/or MEK signalling or receptors such as MET and HER2. These genomic alterations ultimately converge on activation of MAPK signalling and were primarily identified in patients with gastrointestinal cancers, including CRC[217]. Combined inhibition of NTRK and downstream MAPK signalling using a MEK inhibitor, with or without a BRAF or MET inhibitor (in tumours harbouring MET amplifications), is effective in preventing the onset of resistance in PDX models. Clinical signals of activity have also been detected in patients with tumours harbouring these specific alterations detected in ctDNA[217]. The presence of tissue-specific mechanisms of resistance constitutes a potential limitation to the design of tissue-agnostic

biomarker-based studies involving targeted agents, although this challenge can potentially be overcome using powerful translational resources and novel trial designs (BOX 2).

KRAS[G12C] inhibitors

The ability to effectively target activated, oncogenic *KRAS* variants has been a 'Holy Grail' of oncology, given that RAS proteins have been defined as 'undruggable' since 1989 (REF.[218]) and all attempts to functionally inhibit oncogenic RAS signalling using alternative methods have also failed[219]. Oncogenic mutant KRAS proteins typically have altered intrinsic GTPase activity and affinity for GTPase-activating proteins, which determines the persistence of KRAS activation and the ability to promote downstream oncogenic signalling through the MAPK and/or PI3K–AKT signalling pathways[220]. Notably, distinct *KRAS* mutations have been shown to have distinct biochemical consequences, with KRAS[G12D] preferentially activating PI3K–AKT over MAPK signalling relative to the G12C and G12R variants[221–223].

Uniquely, the G12C variant of KRAS presents a cysteine residue that confers a target for selective covalent inhibitors that lock the nucleotide-binding site in its GDP-bound, inactive state[224]. *KRAS*[G12C] accounts for about 11% of all oncogenic *KRAS* mutations in CRCs but up to 44% in NSCLCs[225]. For this reason, the development of selective KRAS[G12C] inhibitors has mainly involved patients with NSCLC, with gradual optimization of the drug properties[226,227]. The first two selective irreversible inhibitors to enter clinical testing, sotorasib (formerly AMG510) and adagrasib (formerly MRTX849), are supported by a strong preclinical rationale, including

investigations of both potential mechanisms of resistance and active combination therapies[228,229]. For example, sotorasib has shown robust preclinical activity in models of *KRAS*[G12C]-mutant cancers, including in PDXs and a syngeneic CT26 model[228]. In phase I trials, patients with NSCLC had an ORR of 54% and no primary progressive disease (DCR 100%), with an excellent safety profile and few drug-related adverse events, with no dose-limiting toxicities even at the highest selected dose of 960 mg[228,230]. Updated results from patients with CRC were reported in September 2020, indicating a low ORR (7.1%), albeit with a clinically relevant DCR (76%), and a clinically meaningful median PFS duration of 4.2 months at the 960 mg dose in this heavily pretreated population[231,232].

Regarding adagrasib, PDX models from various solid tumour types indicate a 65% response rate, although clinical data are available only from a small number of patients with NSCLC or CRC, with partial responses observed in three of five and in one of two patients, respectively[229]. Phase II trials designed to further investigate the efficacy and safety of sotorasib or adagrasib are currently ongoing (NCT04185883, NCT03785249, NCT04330664). A further two *KRAS*[G12C]-selective inhibitors have entered phase I trials, JNJ-74699157 and LY3499446 (REF.[233]) (NCT04006301 and NCT04165031, albeit the latter terminated early in December 2020 owing to an unexpected toxicity finding).

Resistance to KRAS[G12C] inhibitors. The first results from clinical studies revealed a notable difference in response rates to KRAS[G12C] inhibitors in patients with NSCLC compared with those with CRC, which correlates with differences in oncogenic signalling pathways between these two tumour types. Potential mechanisms of primary resistance to KRAS[G12C] inhibitors were researched systematically since the early clinical development of these agents. KRAS signalling is affected by upstream stimuli that lead to alterations in nucleotide binding affinity and GTPase activity, while bypass mechanisms can also lead to resistance and support tumour growth through KRAS-independent mechanisms, including activation of CDK4, CCND1 and AXL signalling[234]. In fact, blockade of collateral signalling pathways using PI3K, mTOR or IGF1R inhibitors increases the efficacy of KRAS[G12C]-selective inhibitors in NSCLC cell lines and PDX models[235,236]. Moreover, downstream KRAS signalling is also regulated by negative feedback mechanisms, such as the induction of phosphatases including dual-specificity phosphatases and inhibitory proteins, such as members of the sprouty and spred families, as previously demonstrated with BRAF and MEK inhibition[237,238]. This observation highlights the importance of targeting the RTK–RAS–MAPK pathway at multiple nodes in order to fully suppress the oncogenic effects of mutant KRAS proteins, including with KRAS[G12C] inhibitors. Reactivation of RTKs and signalling through wild-type KRAS provide a mechanism of pathway adaptation and resistance to selective inhibitors in several different preclinical models of *KRAS*[G12C]-mutant tumours of different histologies. For example, the addition of a SHP2 inhibitor to selective KRAS[G12C] inhibition was able to overcome adaptive feedback resistance to single-agent KRAS inhibition

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Box 2 | Peculiar aspects in the development of therapies for rare targets

The development of inhibitors targeting low-prevalence oncogenic drivers, such as gene fusions, provides an example of the challenges associated with 'precision drug development' in oncology. The tumour-agnostic approval of NTRK inhibitors is an important paradigm shift in the attitudes of regulatory agencies. For decades, targeted drugs have followed the traditional development pathway originally devised for cytotoxic agents, with tumour-agnostic accrual allowed only during the escalation stage of phase I trials, aimed at finding dose-limiting toxicities. Efficacy instead was always explored according to histology, including in phase I–II dose-expansion cohorts. However, basket trials are designed for the opposite purpose, that is, to group patients based upon genetic information — often a particular genetic or genomic alteration. Basket trials have thus become essential when the rarity of the biomarker makes grouping by primary histology highly impractical[276]. The demonstration of efficacy in basket trials depends mostly on targeting a driving genetic abnormality while also considering context specificity[240,277]; hence, the importance of a substantial preclinical body of knowledge. Importantly, exposure in multiple tumour types sharing the same actionable alteration can enable an improved understanding of mechanisms of sensitivity and resistance. The immune-checkpoint inhibitor pembrolizumab was the first anticancer drug approved for an expanded indication (microsatellite instability-high cancers) based only on the presence of a specific genetic alteration, regardless of origin or site of the cancer[278]. Another relevant point regarding targeted therapy for rare gene fusions is the optimization of treatment sequences to overcome acquired resistance within the framework of basket trials. One possible strategy, which was incorporated into the evaluation of the activity of the second-generation NTRK inhibitor selitrectinib (LOXO-195), is the use of single-patient protocols whenever a clinical trial is not logistically feasible[279,280]. In order to be timely and successful, targeted drug development, especially for rare genomic alterations, needs the active involvement and cooperation of many stakeholders, including drug companies, health-care providers and regulatory bodies[280].
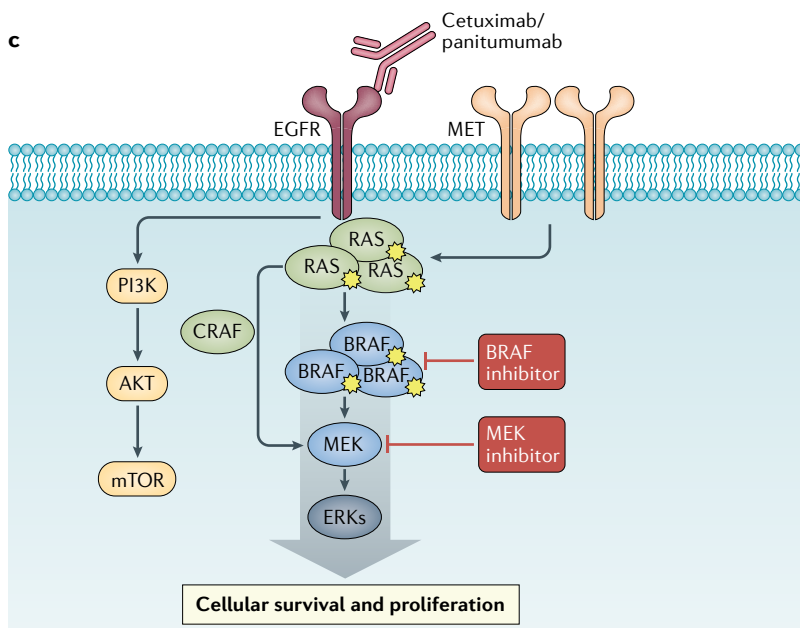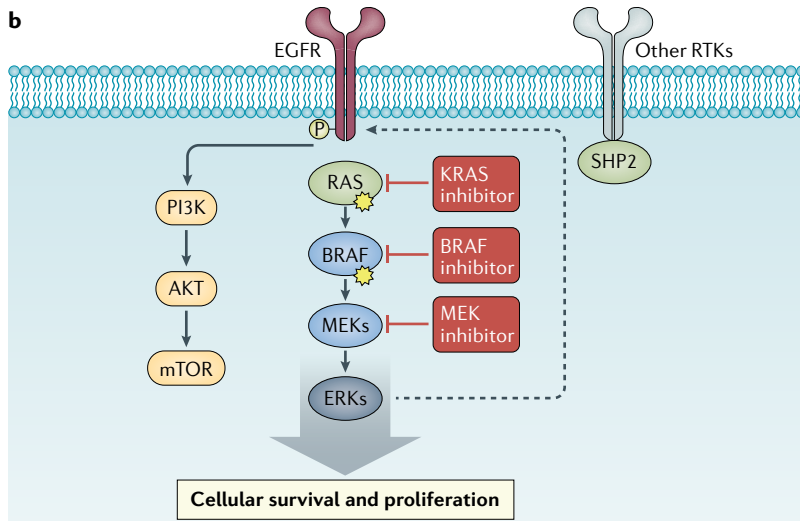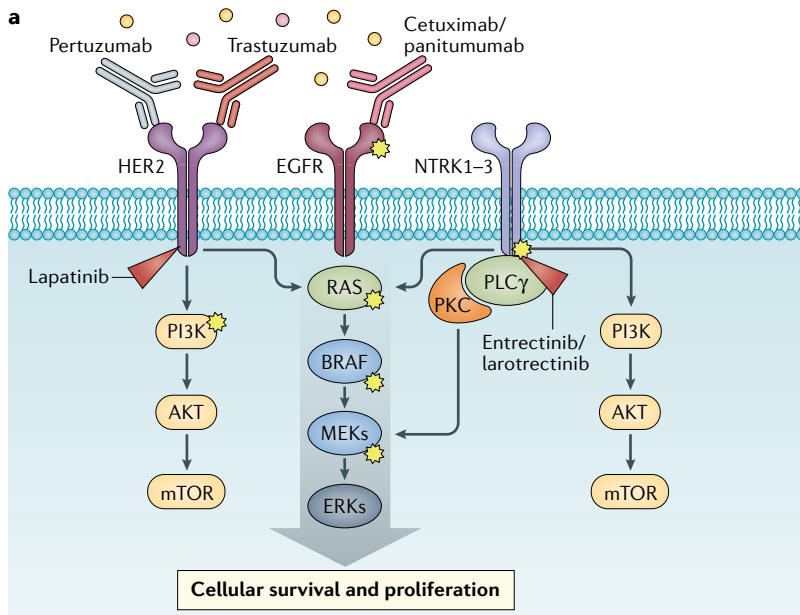
Fig. 3 | **Central role of EGFR–RAS–MAPK signalling in CRC. a** | EGFR-mediated activation of RAS–MAPK signalling drives the proliferation of colorectal cancer (CRC) cells in the absence of downstream activating mutations, and can be attenuated using anti-EGFR antibodies. In tumours bearing oncogenic alterations of HER2 or NTRK1–3, selective inhibition of these receptor tyrosine kinases can quench downstream RAS–MAPK signalling. Additional mutations in the targeted kinases or alterations in the downstream RAS/BRAF/MEK proteins (yellow flashes) are responsible for primary and/or acquired resistance to receptor tyrosine kinase blockade; **b** | In tumours bearing RAS or BRAF oncogenic mutations (yellow flashes), their direct inhibition might result in the reactivation of the EGFR–RAS–MAPK pathway through feedback stimulation of EGFR or other receptor tyrosine kinases, acting as mechanisms of primary resistance. **c** | Mechanisms of acquired resistance to target inhibitor combinations in BRAF-mutant CRC include amplifications of other targets (such as MET) or amplifications and/or mutations in RAS, BRAF and/or MEK (yellow flashes).

across several in vitro and in vivo models[239]. In another example focused on CRC, EGFR reactivation was shown to be the main mediator of resistance to sotorasib, and the vertical suppression of EGFR–MAPK signalling with the combination of cetuximab and sotorasib was able to overcome adaptive resistance, both in cell lines and in PDX models[8].

The experience gathered from attempts to target the MAPK pathway using BRAF and MEK inhibitors in CRC will be key as the efficacy of KRAS^{G12C} inhibitors as monotherapies remains low. Preclinical data are fostering the timely design of clinical trials that integrate biological insights into more effective KRAS blockade in patients with CRC, including trials involving a KRAS^{G12C} inhibitor in combination with a SHP2 inhibitor (NCT04330664) or with the EGFR inhibitors cetuximab or afatinib (NCT03785249).

Future directions

Considering that nearly 50% of all CRCs are driven by 'undruggable' oncogenes of the *RAS* family, progress in targeted therapies for patients with CRC has been limited, relative to the experience with other solid tumours, such as NSCLC or melanoma, for which a relevant subset of patients with metastatic disease are now able to receive targeted agents as first-line therapies. Nonetheless, preclinical models including PDX models, which can be used to generate 'cancer avatars', coupled with liquid biopsy assays, have become an essential proxy to help dissect the complexity of CRCs. These powerful preclinical and co-clinical models have highlighted that the successful personalization of therapy for patients with CRC requires a shift of focus from single genetic biomarkers to dynamic molecular maps charting mechanisms of adaptation and resistance as they evolve[107,240–242]. The pertinent use of specific CRC models is progressively limiting the number of developmental dead ends and reducing the extent of drug attrition prior to clinical development. A good example of this paradigm is provided by the experience with the RTK–RAS–MEK–ERK pathway, which is a major driver of cell proliferation in CRC (FIG. 3). Approved or experimental

drugs targeting many nodes of this pathway are available, including for several RTKs, SHP2, RAF, MEK, ERK and now also specific mutant RAS proteins, namely KRAS[G12C], although preclinical and clinical data suggest that these targeted therapies lead to rapid onset of resistance when administered as monotherapies. To improve efficacy, vertical doublets are being investigated preclinically with promising preliminary results published for combinations such as SHP2 and MEK inhibitors[243] and KRAS[G12C] inhibitors plus anti-EGFR antibodies or SHP2 inhibitors[8,239]. Interestingly, the initial clinical experience with vertical combination therapies at the maximum-tolerated doses resulted in only marginal levels of activity and considerable toxicities[244]. Nonetheless, preclinical data published in 2020 indicate that multiple drugs targeting the same signalling pathway are strikingly effective and well tolerated when combined at low concentrations in preclinical models of *EGFR*-mutant NSCLC and pancreatic cancer[245,246]. This so-called multiple low-dose (MLD) strategy might also alleviate the selective pressures on individual nodes of the pathway and thus avoid the selection of drug-resistant variants. Exploring the MLD strategy as a method of targeting MAPK signalling might provide an attractive path forward in CRC.

Increasing the availability of tumour genotyping in the form of amplicon-based NGS coupled with copy number, gene fusion and outlier gene expression panels, which are now used both in academically driven clinical research (mostly as customized panels) and in industry-sponsored trials (typically as commercial panels) is a crucial step for the integration of genomic biomarkers into routine clinical practice[247]. However, using a multigene panel to select a targeted therapy for a patient with metastatic CRC is still subject to the availability of a treatment targeting that specific alteration[248,249]. Research published in December 2020 demonstrates that the use of ctDNA as the primary source of material for NGS has the potential to increase the trial enrolment rate without compromising treatment efficacy compared with tissue genotyping[26].

A point of possible convergence between preclinical research and the clinical usefulness of a genomic biomarker can be found in the ESMO scale for clinical actionability of molecular targets (ESCAT)[250]. This project is designed to improve the implementation of precision medicine in the clinical management of patients with cancer via standardization of the reporting and interpretation of relevant genomics data, based on clinical actionability. This framework constitutes a potential mechanism for selecting the relevant targets to include in multigene panels in terms of the ability of targeted therapies to improve patient outcomes and provides a common language for all relevant stakeholders, including those involved in cancer medicine and drug development[250].

On the research side, master observational trials (MOTs)[251] offer the unique opportunities of providing the embedded basic research laboratories with biological samples of potentially all types (such as tissue samples, blood samples, peripheral blood mononuclear cells, faeces and several others) both from real-world patients and those enrolled in interventional proof-of-concept trials. AlfaOmega provides an example of such a MOT construct that enables more comprehensive data collection by integrating different biological samples from ongoing clinical trials involving patients with CRC, such as the ARETHUSA and PEGASUS[252,253] trials. Such a pioneering overlay of preclinical and co-clinical research is needed not only for the future development of targeted agents, but also to include other therapeutic strategies, such as those targeting DNA repair and those that promote anticancer immune responses, in the armamentarium for CRC. The observation that MSI is predictive of excellent responses to anti-PD-1 and/or anti-CTLA4 monoclonal antibodies[254,255] highlights how some cancer-specific biomarkers can have substantial effects on how the immune system reacts to the cancer[29]. Nonetheless, an increasing understanding of DNA damage response and repair is enabling the development of novel targeted therapies that selectively affect cancer cells with functionally deficient DNA repair systems[256,257]. Although the utility of such treatment strategies still needs to be established (for example, through the use of patient-derived models), the number of proteins eligible for targeted inhibition is broadening to include molecular pathways not involved in oncogenic signalling but rather in the maintenance of genomic stability[258].

Conclusions

In conclusion, the bidirectional flow of information between preclinical models and patients (the translational workflow) has been proven to be successful in the development and optimization of targeted therapies for patients with CRC. This approach provides an effective paradigm that should guide the development of the next generation of clinical trials, which will ultimately lead to better treatments for patients with CRC.

Published online: 16 April 2021

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