Germline polymorphisms as biomarkers of tumor response in colorectal cancer patients treated with anti-EGFR monoclonal antibodies: a systematic review and meta-analysis

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INTRODUCTION

Colorectal cancer (CRC) is a leading cause of cancer death, with a large fraction of patients developing advanced or metastatic disease.1 For most of these patients, systemic control of disease is paramount, and is now achievable via targeted therapies that directly inhibit molecular drivers of tumor proliferation. By far the most commonly used of these therapies are monoclonal antibodies to the epidermal growth factor receptor (EGFR), which include cetuximab and panitumumab. These drugs not only help to achieve systemic control in metastatic disease after other agents have failed, but also have a much-improved side-effect profile compared to traditional therapies such as irinotecan, oxaliplatin and fluoropyrimidines.2

Because the majority of CRC patients show no response to anti-EGFR monoclonal antibody therapies, considerable efforts are underway to identify up-front the patients who will respond, so that the rest can be spared the time, expense and side effects of an ineffective treatment. One advance in this arena has been the recognition that tumor KRAS mutations are strongly associated with non-response to anti-EGFR drugs,3 since which KRAS testing has become routine. However, even after such testing, more than half of the patients still show no response to anti-EGFR drugs,4,5 indicating a pressing need for additional research. This ongoing effort has led to the discovery and adoption of NRAS/HRAS testing in many jurisdictions,6,7 and has identified some well-studied candidate mutations in genes such as BRAF, PIK3CA and PTEN, whose effectiveness as predictive markers remains uncertain,5,8,9

Genetic alterations with potential as predictive biomarkers in this scenario may affect either tumor (somatic) or patient (germline) DNA. Alterations of somatic tumor DNA (that is, ‘tumor gene mutations’) directly affect tumor cells, and can thus alter tumor characteristics such as growth rate, invasiveness, metastatic potential and vulnerability to particular drugs. In contrast, alterations in germline DNA (that is, ‘genetic polymorphisms’) directly affect patient cells and can thus influence patient factors such as drug bioavailability, kinetics and metabolism, as well as host immune interactions and local tissue responses.

While many germline polymorphisms have been proposed as biomarkers in anti-EGFR monoclonal antibody-treated CRC, studies have often yielded inconsistent results. This may be due to the lack of an underlying true association, heterogeneity of study population or low power in smaller studies. For these reasons, we undertook a systematic review and meta-analysis to evaluate the association of these polymorphisms as putative clinical biomarkers of response to cetuximab/panitumumab therapy. We focused on response as an outcome because (1) response was much more widely and uniformly reported among studies in this area, and (2) the majority of studies did not have control arms of patients not
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Ovid MEDLINE was searched using a date range of January 1990 to September 2015. The search string used was ‘(exp Polymorphism, Genetic/ or polymorphism*;mp.) and (exp Colorectal Neoplasms/ or (colon or rectal or colorectal);mp.) and (neoplas* or tumor* or tumour* or cancer or carcinoma or adenocarcinoma;mp) and (cetuximab or panitumumab';mp.)’.

RESULTS

Systematic review results
The systematic review yielded 87 studies, of which 47 failed to meet the inclusion or exclusion criteria (Figure 1). Of the 40 remaining articles retained for systematic review, 23 were included in the subsequent meta-analysis. Of the 17 excluded studies, seven had insufficient data on covariate or response variables involved polymorphisms only assessed in fewer than three separate studies, and one was excluded for reporting only disease control rate in patients with KRAS mutant (rather than KRAS wild-type) tumors. Of those included in the meta-analysis, one study consisted of new data, published for the first time as part of this meta-analysis, on two of the polymorphisms (FCGR2A and FCGR3A), from analysis of samples from CO17, a Phase III clinical trial of cetuximab versus best supportive care. These 23 studies were included for meta-analysis, and their characteristics are shown in Table 1.

Most studies (57%) used a prospective cohort design, with the remainder using a retrospective cohort design. Most studies (56%) gave anti-EGFR agents as salvage therapy, while a few (22%) gave it as line or neoadjuvant therapy, and the remainder were mixed. KRAS status was unknown in most studies (70%) and was limited to wild type in the remainder. All studies reported response, and most also reported one or more survival outcomes. The geographic location of studies and their ethnic composition were diverse. While most studies investigated survival outcomes, incomplete reporting of these outcomes was common and was a major limitation to the meta-analysis. For example, many studies reported full results (that is, at least an effect size and measure of precision) only for selected polymorphisms, often those that were the most statistically significant. Included studies were subjected to quality review by two independent reviewers. These reviewers demonstrated good agreement with a Spearman correlation coefficient of 0.90, while the mean quality score was 1.96 (range 1.56–2.29), and the category cut-offs were chosen as adequate (< 1.8), good (1.8 ≤ < 2.0), very good (2.0 ≤ < 2.2), and excellent (≥2.2). The resulting quality ratings are also shown in Table 2, along with a summary of which studies investigated which of the polymorphisms that were studied via meta-analysis. The definitions of tumor response used are also shown in Table 2; most were variations of the RECIST criteria.

Meta-analysis of tumor response
Eleven polymorphisms were suitable for meta-analysis (see Table 2). Although some polymorphisms were within genes clearly related to known mechanisms of drug activity (EGF, EGR, FCGR, KRAS polymorphisms), others were relatively tangential (CCND1, COX2, IL8 and VEGF polymorphisms). Most had some data reporting a putative functional consequence of carrying different polymorphic alleles. Among the relevant studies, both prospective and retrospective cohort study designs were well represented, and several were phase II/III trials. The median number of patients per analysis was 110 (range 50–740).

Meta-analysis methodology
Polymorphisms identified in the systematic review were evaluated for their suitability for meta-analysis. Only polymorphisms with published results from at least three separate studies were included. Studies were thus included in the meta-analysis if they (1) reported data on an appropriate polymorphism and (2) reported data in sufficient detail to allow meta-analysis for each outcome. The study methodology was categorized as either prospective or retrospective (depending on the method of cohort recruitment), with notation of whether the cohort was ascertained within a Phase II or randomized clinical trial. For studies with incomplete reporting of detailed results, attempts were made to contact the study authors to obtain complete information. Authors were asked to provide either summary statistics or raw data from which summary statistics (for example, counts) could be calculated.

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For each polymorphism, we calculated a pooled effect with associated standard error, and Higgins’ $I^2$ (see Table 3), and constructed funnel plots (not shown). Two polymorphisms showed pooled relative risks of response that differed significantly from 1.0, with $\alpha = 0.05$: the EGF A61G (rs4444903) and EGFR R497K (rs11543848) polymorphisms. However, neither result retained statistical significance after correction for multiple testing. Among the polymorphisms not associated with outcome were the FCGR2A H131R (rs1801274) and FCGR3A F158V (rs396991), which have garnered much interest and involved the largest number of studies among the polymorphisms reviewed.

A few sensitivity analyses did increase the strength of association for polymorphisms with borderline statistical significance, for instance, to $P = 0.004$ for EGF 61 in one sensitivity analysis; nonetheless, none retained significance at Qo0.05 after correcting for even the baseline level of multiple comparisons among the polymorphisms reviewed.

DISCUSSION
The present systematic review and meta-analysis of 23 eligible studies was able to evaluate pooled effects for 11 polymorphisms on tumor response in CRC patients treated with anti-EGFR monoclonal antibody therapies. Two polymorphisms demonstrated nominal statistical significance, but these associations were not robust to correction for multiple testing.

The published literature studying the association of germline polymorphisms with clinical outcome in anti-EGFR-treated CRC patients presents certain challenges. Most prominently, published studies have historically been quite small, fewer than 150 patients. This has likely been due to logistic difficulties in assembling large patient cohorts from individual centers, the greater cost in dealing with large cohorts, and the fact that, in the past, the number of centers offering anti-EGFR drugs and the number of patients receiving such agents were both small. This combination of multiple groups studying small samples increases the risk that any discovered statistically significant association is in fact false-positive. This is a central problem in all biomedical research, and is due in large part to factors beyond the control of individual researchers.15

One of the main tools available to address this challenge is meta-analysis, which allows evidence to be pooled across studies and can increase the precision of estimates as well as the statistical power to discover true associations, while also showing individual more extreme results in the context of all similar studies.17 In this context, it is expected that many associations showing statistical significance in particular studies will fail to find support after meta-analysis, and that this generally indicates the absence of a relevant underlying association. Indeed, this same trend has been observed in recent large, carefully conducted studies on certain polymorphisms that failed to replicate significant associations observed in smaller prior studies.18,19

However, negative meta-analysis results must be taken with important caveats including study and patient heterogeneity, low minor allele frequencies (limiting meta-analysis study power), low response rates to anti-EGFR agents, inappropriate use of response rate as a surrogate for survival in this context, trade-offs in...
### Table 1. Summary of studies eligible for meta analysis, grouped by study methodology

| Author          | Year | Region                  | Study methodology | N  | Intent of therapy | Other chemotherapy | KRAS status | Reported outcomes |
|-----------------|------|-------------------------|-------------------|----|-------------------|---------------------|-------------|-------------------|
| Saridaki        | 2014 | Belgium, France, USA    | Mixed             | 559| Mixed             | Mixed               | Unselected  | Response, PFS, OS |
| Loupakis        | 2014 | California              | Prospective cohort| 113| Mixed             | Irinotecan          | Wild type   | Response, PFS, OS |
| Graziano        | 2008 | Italy                   | Prospective cohort| 71 | Salvage           | Irinotecan          | Unselected  | Response, PFS, OS |
| Inoue           | 2014 | Japan                   | Prospective cohort| 57 | Salvage           | Mixed               | Wild type   | Response, PFS, OS |
| Sclafani        | 2014 | Response, PFS, OS       | Prospective cohort| 105| Adjuvant          | Cetuximab, oxaliplatin | Wild type   | Response, PFS, OS |
| Ettienne-Grimaldi | 2012 | France                  | Prospective cohort| 52 | First-line       | Irinotecan, folinic acid, UFT | Unselected  | Response, OS     |
| Lurje           | 2008 | California              | Prospective cohort| 130| Surgery           | None                | Unselected  | Response, PFS, OS |
| Zhang           | 2010 | California              | Prospective cohort| 65 | Salvage           | Mixed               | Unselected  | Response, TTP, OS |
| Zhang           | 2011 | California              | Prospective cohort| 111| Salvage           | None                | Unselected  | Response, PFS, OS |
| Kjersem        | 2012 | Norway                  | Prospective cohort| 180| First-line       | Oxaliplatin, folinic acid, UFT | Unselected  | Response, PFS, OS |
| Kjersem        | 2014 | Norway                  | Prospective cohort| 504| First-line       | Oxaliplatin, folinic acid, UFT | Unselected  | Response, PFS, OS |
| Jonker          | 2015 | Canada and Australia    | Prospective cohort| 138| Salvage           | None                | Unselected  | Response, PFS, OS |
| Hsieh           | 2012 | Taiwan                  | Retrospective cohort| 118| First-line       | Oxaliplatin, folinic acid, UFT | Wild type   | Response, PFS, OS |
| Zhang           | 2010 | France                  | Retrospective cohort| 69 | Mixed             | Mixed               | Unselected  | Response, PFS, OS |
| Zhang           | 2011 | France                  | Retrospective cohort| 58 | Salvage           | Irinotecan          | Unselected  | Response, TTP, OS |
| Paez            | 2012 | Korea                   | Retrospective cohort| 118| Mixed             | Mixed               | Unselected  | Response, PFS, OS |
| Sebio           | 2013 | Spain                   | Retrospective cohort| 104| Salvage           | Mixed               | Unselected  | Response, PFS     |
| Geva            | 2015 | Europe (multicenter)    | Retrospective cohort| 740| Salvage           | Mixed               | Wild type   | Response, PFS, OS |

**Abbreviations:** C/P, patients received either cetuximab or panitumumab; DCR, disease control rate; DSS, disease-free survival; LA, patients had locally advanced disease; N, number of participants; OS, overall survival; PFS, progression-free survival; TTP, time to progression; UFT, Tegafur/uracil.

### Table 2. Matrix illustrating, for each study, which polymorphisms it investigated, its quality rating and its stated definition of response

| Study reference | FCGR2A | FCGR3A | EGFR | EGFR 3'UTR | KRAS | CCND1 | VEGF | COX2 | IL-8 | Study quality | Definition of response |
|-----------------|--------|--------|------|------------|------|-------|------|------|------|---------------|-------------------------|
| Saridaki, 2014  | X      |        |      |            |      |       |      |      |      | Good          | Objective respiratory rate |
| Loupakis, 2014  |        |        |      |            |      |       |      |      |      | Very good      | RECIST 1.0               |
| Graziano, 2008  | X      |        |      |            |      |       |      |      |      | Very good      | RECIST 1.0               |
| Inoue, 2014     | X      |        |      |            |      |       |      |      |      | Good           | RECIST                  |
| Sclafani, 2014  | X      |        |      |            |      |       |      |      |      | Very good      | WHO criteria, modified    |
| Ettienne-Grimaldi | 2012 | X      | X    |            |      |       |      |      |      | Good           | RECIST                  |
| Lurje, 2008    | X      | X      |      |            |      |       |      |      |      | Adequate       | RECIST                  |
| Zhang, 2010    | X      | X      |      |            |      |       |      |      |      | Good           | RECIST                  |
| Zhang, 2011    | X      |        |      |            |      |       |      |      |      | Very good      | WHO criteria, modified    |
| Kjersem, 2012  | X      |        |      |            |      |       |      |      |      | Good           | RECIST                  |
| Kjersem, 2014  | X      |        |      |            |      |       |      |      |      | Excluded*      | RECIST 1.0               |
| Hsieh, 2012    | X      |        |      |            |      |       |      |      |      | Good           | RECIST 1.1               |
| Negri, 2014    |        |        |      |            |      |       |      |      |      | Good           | RECIST                  |
| Calemma, 2012  | X      |        |      |            |      |       |      |      |      | Good           | RECIST                  |
| Hu-Lieskovian, 2015 | X      | X      | X    |            |      |       |      |      |      | Good           | RECIST 1.0               |
| Bubeau, 2009  | X      |        |      |            |      |       |      |      |      | Good           | RECIST 1.0               |
| Dahan, 2011    | X      | X      |      |            |      |       |      |      |      | Good           | RECIST, modified          |
| Park, 2012     | X      |        |      |            |      |       |      |      |      | Very good      | RECIST 1.1               |
| Paez, 2010     | X      |        |      |            |      |       |      |      |      | Good           | RECIST 1.0               |
| Sebio, 2013    | X      |        |      |            |      |       |      |      |      | Very good      | RECIST 1.0 or WHO        |

*Jonker (2007) was excluded because no appropriate polymorphism-related manuscript was available, and because both quality reviewers were involved with the study.
tried. Studies also varied in whether patients were taking anti-EGFR therapy, might have been used, and which regimens had been previously used for surgery or chemotherapy, how many prior lines of chemotherapy for substantial variation. Patients varied in whether they had prior morphisms with response to anti-EGFR therapy, there was room included study designs. While all studies enrolled patients with CRC (usually metastatic) and evaluated the association of poly-

An important limitation in this study was the heterogeneity of statistical modeling, and poor reporting of outcomes in the published literature. We address each of these issues below.

**Study heterogeneity**
An important limitation in this study was the heterogeneity of included study designs. While all studies enrolled patients with CRC (usually metastatic) and evaluated the association of polymorphisms with response to anti-EGFR therapy, there was room for substantial variation. Patients varied in whether they had prior surgery or chemotherapy, how many prior lines of chemotherapy might have been used, and which regimens had been previously tried. Studies also varied in whether patients were taking anti-EGFR drugs as monotherapy or along with other drugs, and also in the choice of any such drugs. The criteria employed for evaluating tumor response varied (see Table 2). The Kras status of study patients (and indeed, whether Kras testing was performed at all) also varied, with many studies of all patients regardless of Kras status, and several others with only Kras wild-type tumors. Finally, the country of recruitment and thus ethnicity distribution of patients varied considerably, with potential masking of significant associations that may be present only within particular ethnic groups. These differences represent a limitation that is difficult to fully resolve when the literature typically includes only three to six studies per polymorphism, which is too small a number for meta-regression.

However, as an alternate approach to address these sources of heterogeneity, along with the possibility that they may have led to false-negative results, we performed multiple sensitivity analyses (see Results section), some of which produced lower nominal P-values, but none of which were robust to correction for multiple testing, rendering them unlikely to be of great promise. Of course, it is difficult to entirely exclude the possibility that a relevant, statistically significant result may be recoverable for one or more polymorphisms within some alternate subgroup of studies. However, an exhaustive search for such associations would also undoubtedly reveal many more false positives than true associations.

**Statistical modeling tradeoffs**
We chose the more conservative random-effects model for meta-analysis rather than a fixed-effects model. While this may reduce the study's ability to identify true associations, the random-effects model is more suited to situations with heterogeneity between study designs, which is certainly abundant in this case.

We corrected for multiple comparisons relating to two different factors: (1) the multiple polymorphisms being tested and (2) the multiple genetic models being tested per polymorphism. However, different genetic models do not represent independent statistical tests, since there is a strong correlation between the results of recessive, dominant and additive models for a given allele. Thus, typical methods of correction, which assume independence of statistical tests, run the risk of being too conservative. We addressed this concern in two ways. First, we chose to limit the false discovery rate to Q < 0.05 using the well-known Benjamini–Hochberg procedure. Second, realizing that even this procedure could yield overly stringent results for the stated false discovery rate in the setting of dependent tests, we performed a sensitivity analysis at a more liberal rate of Q < 0.2, which still failed to show any significant results.

Finally, it could be argued that our approach to meta-analysis, which included separate tests for each of three genetic models, is less likely to find true associations due to the necessary correction for multiple testing. This argument would posit that, had we instead chosen a single genetic model for each polymorphism based on prior publications, a significant association might have been found. To address this concern, we simulated perfect foreknowledge by picking the lowest P-value model for each polymorphism and correcting for multiple testing using only those 11 models. The conclusions were unchanged, with no model retaining significance at the chosen cutoff of Q < 0.05. Even on extending this approach of perfect foreknowledge further to allow selection of the lowest P-value model for each polymorphism including all sensitivity analyses, conclusions remained unchanged, with no model retaining significance.

**Limitations of tumor response as an outcome**
An important limitation of analyses of tumor response in cetuximab-treated colorectal carcinoma is the low rates of tumor response in the published literature. In CO17, the study that originally demonstrated cetuximab efficacy in metastatic CRC, the response rate in the cetuximab treatment group was only 8%. This introduces a challenge with statistical power—even assuming that a beneficial effect exists for certain polymorphisms, this low rate would make it difficult to demonstrate. This issue is beginning to be addressed by new larger studies, as well as by the present meta-analysis.

However, an even more fundamental point relates to the hypothesized action of anti-EGFR agents in CRC. The low observed

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**Table 3.** Meta-analysis results for polymorphisms with at least three studies

| Polymorphism | OMIM no. | RS no. | Test allele (model) | RR (95% CI) | N | Higgins’ I (%) | P-value | Q-value |
|--------------|----------|--------|---------------------|-------------|---|----------------|---------|---------|
| CCND1 870 A>G | 168461 | rs17852153 | A (Recessive) | 1.14 (0.64, 2.04) | 5 | 34.8 | 0.652 | 0.530 |
| COX2 – 765 G>C | 600262 | rs20417 | C (Recessive) | 2.67 (0.69, 10.36) | 3 | 55.0 | 0.155 | 0.427 |
| EGF 61 A>G | 131530 | rs4444903 | G (Recessive) | 1.81 (1.08, 3.02) | 6 | 48.4 | 0.023 | 0.257 |
| EGFR – 216 G>T | 131550 | rs712829 | T (Recessive) | 1.27 (0.78, 2.06) | 3 | 0.0 | 0.331 | 0.460 |
| EGFR 497 R>K | 131550 | rs11543848 | K (Recessive) | 1.52 (1.01, 2.31) | 6 | 0.0 | 0.047 | 0.460 |
| EGFR 3’UTR (CA)n S>L | 131550 | N/A | S (Recessive) | 1.23 (0.81, 1.85) | 7 | 45.4 | 0.334 | 0.259 |
| FCGRA2 131 R=H | 146790 | rs1801274 | R (Dominant) | 1.09 (0.94, 1.27) | 15 | 0.0 | 0.251 | 0.275 |
| FCGRA3A 158 F=V | 146740 | rs396991 | V (Recessive) | 1.03 (0.85, 1.24) | 15 | 0.0 | 0.781 | 0.781 |
| IL8 – 251 T>A | 146930 | rs4073 | T (Recessive) | 1.29 (0.78, 2.13) | 3 | 0.0 | 0.324 | 0.530 |
| KRAS Let-7 T>G | 190070 | rs61764370 | G (Dominant) | 1.36 (0.70, 2.65) | 5 | 83.2 | 0.370 | 0.460 |
| VEGFA 936 C>T | 192240 | rs3025039 | T (Dominant) | 1.34 (0.81, 2.21) | 3 | 0.0% | 0.710 | 0.460 |

Abbreviations: A/C/G/T, respective oligonucleotides; CI, confidence interval; RR, relative risk; S, the shorter number of CA repeats (with the other allele being L, the longer number of CA repeats); OMIM, online mendelian inheritance in man database; RS, reference single nucleotide polymorphism cluster identifier. The recessive model had a slightly lower P-value for this polymorphism, but the dominant model was chosen to include the largest number of high-quality studies, not all of which reported sufficient data for analysis of all allele combinations. Each polymorphism’s common name is listed along with the corresponding OMIM gene number and dbSNP polymorphism number. For each polymorphism, the results of meta-analysis are presented for the genetic model resulting in the lowest P-value. Data presented for each analysis included the pooled relative risk with the corresponding confidence interval, number of studies contributing data to the analysis, Higgins’ I², P-value, and false discovery rate Q-value.

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response rate may be a sign that the beneficial effects of cetuximab on survival (which is ultimately the most important outcome) are largely independent of tumor response. For example, if the mechanism was primarily inhibition of tumor growth (or antiproliferation, given that EGFR acts on cellular growth) rather than inducing tumor shrinkage, then there may be a true benefit in the absence of any significant tumor response. It would thus be ideal to also perform a meta-analysis of patient survival in this clinical area. Unfortunately, our ability to do this was limited by inconsistent reporting of outcomes among reviewed studies, which is discussed below.

Inconsistent outcome reporting in published studies

Many reviewed studies reported survival outcomes, such as progression-free survival and overall survival, in addition to tumor response. Survival outcomes are of prime importance in evaluating oncology therapeutics, including anti-EGFR therapies, and would ideally be included in this study. Indeed, tumor response is often viewed as a surrogate for survival, which is the outcome of ultimate importance (at least for drug effectiveness; for efficacy, in contrast, tumor response may sometimes be the preferred outcome). Unfortunately, it was not possible to perform a meta-analysis of survival in the present study due to incomplete and/or irregular reporting of survival outcomes. Some studies did not investigate survival outcomes; many others did, but reported effect sizes and precisions for only selected analyses. Even when survival data were reported, its format was inconsistent, variably couched as hazard ratios, Kaplan–Meier plots, P-values for logrank tests or median survivals by patient group. Such problems are routine in the published survival literature, and represent a significant challenge to meta-analysis generally. This was particularly true as we also attempted to contact individual studies to request primary source data and/or re-analyzed the data according to a single standard, which met with only marginal success. In the present study, meta-analysis was technically possible for selected polymorphisms (that is, more than two studies reporting adequate survival outcomes for a given polymorphism); however, given the large proportion of relevant published results that would have been excluded due to inadequate reporting, the potential for biased and misleading results would be extreme. Consequently, the present study is necessarily limited to the more proximal outcome of tumor response, and a meta-analysis of survival outcomes must await more uniform reporting, more widespread sharing of unpublished data or methodological advances that allow the incorporation of studies with incomplete data.

CONCLUSIONS

The present study represents the first systematic review and meta-analysis of germline polymorphisms as biomarkers of tumor response in CRC patients treated with anti-EGFR monoclonal antibody therapy. The resulting pooled analysis, which was possible for 11 of the reviewed polymorphisms, revealed no statistically significant associations after correction for multiple testing. Given the substantial heterogeneity in methodology among included studies, the relatively small numbers of analyzable studies for each polymorphism and the inability to systematically analyze the survival outcomes, this result cannot definitively exclude the possibility of a significant association for one of the included polymorphisms. Nonetheless, these findings were robust to multiple sensitivity analyses, and also parallel an observed trend in recent large, well-conducted studies in the area that have failed to replicate significant associations observed in smaller prior studies.

Equally important in this study is how the results serve to highlight important issues for future research in this area. The present results argue for the use of potentially more fruitful approaches through the planning of larger studies, potentially within consortia to leverage the resources of multiple centers, and, where possible, in adopting an unbiased, genome-wide approach to biomarker discovery that will better facilitate data-sharing, patient-level meta-analysis, and validation of polymorphisms proposed by other groups. Finally, the current literature is quite variable in the reporting of survival outcomes, and it is crucial that future studies publish uniform data regarding all major clinical outcomes for all studied polymorphisms (at least as supplementary data), in order to minimize publication bias and facilitate aggregation of study results, which will be indispensable to future progress in this area.

CONFLICT OF INTEREST

H-JL: advisory board member for Merck KG, Bristol Myers Squibb clinical trial support, Merck Serono and Bristol Myers Squibb. GM: Merck Serono honorarium. JZ: travel and research support to Merck Serona. CSM: advisory board member for Amgen and Merck Serono. AD: advisory board member for Roche, Astra Zeneca, Novartis, Pfizer and Merck Serono. The remaining authors declare no conflict of interest.

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APPENDIX 1

Criteria used for quality review of included articles

| Category | Criteria Summary |
|----------|------------------|
| title and abstract | design in title or abstract; informative/balanced summary in abstract |
| intro - rationale | explain scientific background and rationale |
| intro - objectives | specific objectives; first report vs replication |
| meth - setting | setting, location, dates |
| meth - participants | eligibility criteria, sources, selection methods |
| meth - variables | outcomes, covariates, variants (standard nomenclature), ethnic confounding |
| meth - measurement | lab methods, source/storage of DNA, genotyping method, allele calling algorithm, error rates, call rates, laboratory identified |
| meth - size | how was study size arrived at |
| meth - Q var | describe how quantitative variables were handled in analysis (choice of groupings) |
| Stats | describe all statistical methods, including confounding; software, version, options |

Polymorphism meta-analysis for anti-EGFR monoclonal antibody response

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res - participants
numbers of individuals at each stage of study, reasons for non-participation; number successfully genotyped?

res - descriptive
participant characteristics, information on exposures and potential confounders; number of participants with missing data for variables of interest; follow-up time

res - outcome
outcomes by genotype over time (cohort); summary of outcomes by genotype (case-control)

res - main
unadjusted and (IA) adjusted estimates (which covariates?), precisions; report category boundaries if discretized; results of multiple comparisons adjustments

res - other
summarize results from all variants analyzed; (IA) how can more detailed results be accessed?

disc - results
summarize key results with relation to objectives

disc - limits
study limitations discussed (bias, imprecision, direction/magnitude of bias)

desc - interp
give cautious interpretation considering limitations, multiple testing, other studies

desc - general
discuss generalizability (external validity)

other - funds
give sources of funding and role of funders in present study and (IA) for original studies