

patients with metastatic, refractory or recurrent disease of advanced adenocarcinoma of the stomach or the lower esophagus proven by histology.

In this phase IIa trial the investigational drug was applied in three cohorts, which were recruited sequentially. A first cohort of three patients received repeated doses of IMAB362 at a lower dose level (300 mg/m² body surface area). The antibody was given as a 2 h intravenous infusion. Since no indication for IMAB362-related toxicity was detected in the first cohort, the IMAB362 dose of the second cohort (three patients) was increased to 600 mg/m² body surface area. In a third cohort 19 patients were allocated with the same dose (repetitive application of 600 mg/m² body surface area). Patient samples from this cohort were analyzed for several accompanying analytics i.e. ADCC, CDC, immunophenotyping and genetic immune polymorphisms. All patients of all cohorts have received repeated doses of IMAB362 every two weeks on visits 2, 5, 6, 7 and 8 (5 applications).

The discrepancy of antigen positive tumors (overexpressing the target antigen to similar extent) with regard to responsiveness to intervention with therapeutic monoclonal antibodies such as IMAB362 suggests that there are additional factors which are associated with therapy outcome. This demands careful selection of patients who may have a benefit from antibody therapy.

Therefore, there is a need to develop a test to measure the eligibility of patients for antibody therapy. The present invention addresses this need by providing markers which are associated with favorable and unfavorable outcomes, respectively, in antibody therapy. Furthermore, the present invention demonstrates that these markers are useful as markers for prognosing clinical outcome for cancer patients.

The findings presented herein may be used to select a suitable treatment for a cancer patient and, in particular, to decide whether antibody therapy should be administered to a cancer patient.

SUMMARY

The present invention provides methods of SNP (single-nucleotide polymorphism) genotyping, such as for use in evaluating an individual's likelihood of responding to a

therapeutic treatment for cancer, in selecting a treatment or preventive regimen (e.g., in deciding whether or not to administer a particular therapeutic agent to an individual having cancer, or who is at increased risk for developing cancer in the future), or in evaluating an individual's prognosis for disease severity and recovery.

The present invention is based on the finding that certain genotypes for SNPs are associated with sensitivity/insensitivity of cancer towards antibody treatment such as treatment of CLDN18.2 positive cancer, in particular CLDN18.2 positive gastroesophageal cancer with IMAB362. The present invention is further based on the finding that certain genotypes for SNPs are associated with clinical outcome for cancer patients and thus are useful for prognosing cancer.

Therefore, in one aspect, the present invention is directed to a method of assessing (i) if a cancer patient having a tumor antigen-positive tumor is a responder to treatment with an antibody against the tumor antigen, and/or (ii) if a cancer patient having a tumor antigen-positive tumor, will experience progression-free survival, wherein the tumor antigen is the CLDN18.2 protein; said method comprising determining the genotype for one or more single-nucleotide polymorphisms selected from the group consisting of MUC1 rs4072037, IL-10 rs1800896, DNMT3A rs1550117, SMAD4 rs12456284, EGF rs4444903, CDH1 rs16260, and ERCC1 rs11615 in a sample obtained from the patient. In another aspect, the present invention is directed to the use of an antibody binding to CLDN18.2 for the preparation of a medicament for use in a method of treatment of a cancer patient having a tumor which is positive for CLDN18.2, wherein the patient is characterized by having a reduced risk for not being a responder to treatment with the antibody, wherein the reduced risk is indicated in a tumor sample obtained from the patient by (a) the presence of the homozygous MUC1 rs4072037 [AA] genotype; (b) the presence of the homozygous IL-10 rs1800896 [GG] genotype; (c) the presence of the heterozygous DNMT3A rs1550117 [GA] genotype; (d) the presence of the heterozygous SMAD4 rs12456284 [GA] genotype; (e) the presence of the homozygous EGF rs4444903 [AA] genotype; (f) the presence of the homozygous CDH1 rs16260 [AA] genotype; and (g) the presence of the homozygous ERCC1 rs11615 [TT] genotype.

Also disclosed herein is a method of assessing

(i) if a cancer patient having a tumor antigen-positive tumor is a responder to treatment with an antibody against the tumor antigen, and/or

(ii) if a cancer patient, preferably a cancer patient having a tumor antigen-positive tumor, will experience progression-free survival,

said method comprising determining the genotype for one or more single-nucleotide polymorphisms selected from the group consisting of FCGR2A rs1801274, MUC1 rs4072037, IL-10 rs1800896, DNMT3A rs1550117, SMAD4 rs12456284, EGF rs4444903, CDH1 rs16260, ERCC1 rs11615, and FCGR3A rs396991 in a sample obtained from the patient.

In one example, the presence of the heterozygous FCGR2A rs1801274 [CT] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous FCGR2A rs1801274 [TT] genotype and/or the homozygous FCGR2A rs1801274 [CC] genotype indicates an increased risk of a cancer patient not being a responder to treatment with the antibody and/or an increased risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous MUC1 rs4072037 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous MUC1 rs4072037 [GG] genotype indicates an increased risk of a cancer patient not being a responder to treatment with the antibody and/or an increased risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous IL-10 rs1800896 [GG] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the heterozygous DNMT3A rs1550117 [GA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the heterozygous SMAD4 rs12456284 [GA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous EGF rs4444903 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous CDH1 rs16260 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous ERCC1 rs11615 [TT] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the heterozygous FCGR3A rs396991 [TG] genotype and/or the homozygous FCGR3A rs396991 [TT] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous FCGR3A rs396991 [GG] genotype indicates an increased risk of a cancer patient not being a responder to treatment with the antibody and/or an increased risk of a cancer patient not experiencing progression-free survival.

In one example, the tumor antigen is the CLDN18.2 protein.

Also disclosed herein is a method of assessing

(i) if a cancer patient having a CLDN18.2-positive tumor is a responder to treatment with an antibody against the CLDN18.2 protein, and/or

(ii) if a cancer patient, preferably a cancer patient having a CLDN18.2-positive tumor, will experience progression-free survival,

said method comprising determining the genotype for one or more single-nucleotide polymorphisms selected from the group consisting of FCGR2A rs1801274, MUC1 rs4072037, IL-10 rs1800896, DNMT3A rs1550117, SMAD4 rs12456284, EGF rs4444903, CDH1 rs16260, ERCC1 rs11615, and FCGR3A rs396991 in a sample obtained from the patient.

In one example, the presence of the heterozygous FCGR2A rs1801274 [CT] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous FCGR2A rs1801274 [TT] genotype and/or the homozygous FCGR2A rs1801274 [CC] genotype indicates an increased risk of a cancer patient not being a responder to treatment with the antibody and/or an increased risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous MUC1 rs4072037 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous MUC1 rs4072037 [GG] genotype indicates an increased risk of a cancer patient not being a responder to treatment with the antibody and/or an increased risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous IL-10 rs1800896 [GG] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the heterozygous DNMT3A rs1550117 [GA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the heterozygous SMAD4 rs12456284 [GA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous EGF rs4444903 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous CDH1 rs16260 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous ERCC1 rs11615 [TT] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the heterozygous FCGR3A rs396991 [TG] genotype and/or the homozygous FCGR3A rs396991 [TT] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

In one example, the presence of the homozygous FCGR3A rs396991 [GG] genotype indicates an increased risk of a cancer patient not being a responder to treatment with the antibody and/or an increased risk of a cancer patient not experiencing progression-free survival.

In one case applying to all examples described herein, the antibody acts through recruiting the patient's immune system to destroy tumor cells. In one example, the antibody acts through antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). In one example, the antibody is a monoclonal antibody. In one case applying to all examples described herein, the antibody comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or 51 or a fragment thereof and a light chain comprising an amino acid sequence represented by SEQ ID NO: 24 or a fragment thereof.

In one case applying to all examples described herein, non-responsiveness to treatment with the antibody comprises a relative reduction in one or more of survival, progression-free survival, recurrence-free survival, distant recurrence-free survival, and stable disease.

Also disclosed herein is a method of treating a cancer patient, said method comprising

- a. assessing if the cancer patient is a responder to treatment with an antibody by the method of the invention and
- b. (i) treating the cancer patient with an antibody if the patient has a reduced risk for not being a responder to treatment with the antibody or (ii) not treating the cancer patient with an antibody and/or treating the cancer patient with a treatment regimen which comprises a treatment which is different from a treatment with an antibody if the patient has an increased risk for not being a responder to treatment with the antibody.

In one example, the treatment regimen comprises a treatment not being dependent on the immune system of the patient. In one example, the treatment regimen does not comprise a treatment with an antibody acting through recruiting the patient's immune system to destroy tumor cells. In one example, the treatment regimen comprises surgery, chemotherapy and/or radiation. In one example, the treatment regimen comprises a treatment with a small molecule inhibitor of the tumor antigen and/or an antibody-drug conjugate wherein the antibody is directed against the tumor antigen. In one example, the antibody-drug conjugate is an antibody coupled to a radioactive, chemotherapeutic or toxin moiety. In one example, the antibody-drug conjugate is an antibody coupled to a cytostatic or cytotoxic compound.

Also described herein is a method of assessing the clinical outcome for a cancer patient, said method comprising determining the genotype for one or more single-nucleotide polymorphisms selected from the group consisting of FCGR2A rs1801274, MUC1 rs4072037, IL-10 rs1800896, DNMT3A rs1550117, SMAD4 rs12456284, EGF rs4444903, CDH1 rs16260, ERCC1 rs11615, and FCGR3A rs396991 in a sample obtained from the patient.

In one example, the presence of the heterozygous FCGR2A rs1801274 [CT] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the homozygous FCGR2A rs1801274 [TT] genotype and/or the homozygous FCGR2A rs1801274 [CC] genotype indicates an increased risk of poor clinical outcome.

In one example, the presence of the homozygous MUC1 rs4072037 [AA] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the homozygous MUC1 rs4072037 [GG] genotype indicates an increased risk of poor clinical outcome.

In one example, the presence of the homozygous IL-10 rs1800896 [GG] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the heterozygous DNMT3A rs1550117 [GA] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the heterozygous SMAD4 rs12456284 [GA] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the homozygous EGF rs4444903 [AA] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the homozygous CDH1 rs16260 [AA] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the homozygous ERCC1 rs11615 [TT] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the heterozygous FCGR3A rs396991 [TG] genotype and/or the homozygous FCGR3A rs396991 [TT] genotype indicates a reduced risk of poor clinical outcome.

In one example, the presence of the homozygous FCGR3A rs396991 [GG] genotype indicates an increased risk of poor clinical outcome.

In one example, assessing the clinical outcome for a cancer patient comprises predicting the likelihood of one or more of survival, progression-free survival, recurrence-free survival, distant recurrence-free survival and stable disease. In one example, poor clinical outcome comprises a relative reduction in one or more of survival, progression-free survival, recurrence-free survival, distant recurrence-free survival and stable disease.

In one example, the patient has a tumor antigen-positive tumor and receives a treatment with an antibody against the tumor antigen.

In one case applying to all examples described herein, the sample is a sample comprising DNA. In one example, the DNA has been extracted from a bodily sample of the patient. In one example, the DNA has been extracted from blood.

In one case applying to all examples described herein, the tumor is a solid tumor. In one example, the tumor is a gastroesophageal tumor. In one example, the tumor is an advanced adenocarcinoma of the stomach or the lower esophagus. In one example, the cancer is gastroesophageal cancer. In one example, the cancer is an advanced adenocarcinoma of the stomach or the lower esophagus.

Also described herein is a kit comprising means for determining the genotype for one or more single-nucleotide polymorphisms selected from the group consisting of FCGR2A rs1801274, MUC1 rs4072037, IL-10 rs1800896, DNMT3A rs1550117, SMAD4 rs12456284, EGF rs4444903, CDH1 rs16260, ERCC1 rs11615, and FCGR3A rs396991 in a sample obtained from the patient. In one example, said kit is useful for conducting the methods of all aspects of the present invention. In one example, said kit further comprises a data carrier. In one preferred example, said data carrier is an electronical or a non-electronical data carrier. In one example, said data carrier comprises instructions on how to carry out the methods of all aspects of the invention.

Other objects, advantages and features of the present invention will become apparent from the following detailed description when considered in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Single nucleotide polymorphisms with a statistically significant genotype frequency shift between patient and control population (χ^2 -test, $p < 0.05$).

Assignment of SNP-specific genotypes to bar sections is indicated. Pat. Patient population, Co. Control population.

Figure 2: Relative frequency of homozygous risk genotypes per patient in relation to the number of investigated SNP risk factors per patient. Patients are sorted by increasing frequency of accumulated homozygous risk factors.

Figure 3: Progression-free survival of PP patients differentiated by rs1801274 (FCGR2A) genotype (Kaplan-Meier curve)

CLAIMS

1. A method of assessing

(i) if a cancer patient having a tumor antigen-positive tumor is a responder to treatment with an antibody against the tumor antigen, and/or

(ii) if a cancer patient having a tumor antigen-positive tumor, will experience progression-free survival,

wherein the tumor antigen is the CLDN18.2 protein;

said method comprising determining the genotype for one or more single-nucleotide polymorphisms selected from the group consisting of MUC1 rs4072037, IL-10 rs1800896, DNMT3A rs1550117, SMAD4 rs12456284, EGF rs4444903, CDH1 rs16260, and ERCC1 rs11615 in a sample obtained from the patient.

2. The method of claim 1 wherein

(a) the presence of the homozygous MUC1 rs4072037 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival;

(b) the presence of the homozygous MUC1 rs4072037 [GG] genotype indicates an increased risk of a cancer patient not being a responder to treatment with the antibody and/or an increased risk of a cancer patient not experiencing progression-free survival;

(c) the presence of the homozygous IL-10 rs1800896 [GG] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival;

(d) the presence of the heterozygous DNMT3A rs1550117 [GA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival;

(e) the presence of the heterozygous SMAD4 rs12456284 [GA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival;

(f) the presence of the homozygous EGF rs4444903 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival;

(g) the presence of the homozygous CDH1 rs16260 [AA] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival; and

(h) the presence of the homozygous ERCC1 rs11615 [TT] genotype indicates a reduced risk of a cancer patient not being a responder to treatment with the antibody and/or a reduced risk of a cancer patient not experiencing progression-free survival.

3. The method of claim 1 or 2 wherein the antibody acts through recruiting the patient's immune system to destroy tumor cells, preferably through antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC).

4. The method of any one of claims 1 to 3 wherein the antibody comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or 51 or a fragment thereof and a light chain comprising an amino acid sequence represented by SEQ ID NO: 24 or a fragment thereof.

5. Use of an antibody binding to CLDN18.2 for the preparation of a medicament for use in a method of treatment of a cancer patient having a tumor which is positive for CLDN18.2, wherein the patient is characterized by having a reduced risk for not being a responder to treatment with the antibody, wherein the reduced risk is indicated in a tumor sample obtained from the patient by

- (a) the presence of the homozygous MUC1 rs4072037 [AA] genotype;
- (b) the presence of the homozygous IL-10 rs1800896 [GG] genotype;
- (c) the presence of the heterozygous DNMT3A rs1550117 [GA] genotype;
- (d) the presence of the heterozygous SMAD4 rs12456284 [GA] genotype;
- (e) the presence of the homozygous EGF rs4444903 [AA] genotype;
- (f) the presence of the homozygous CDH1 rs16260 [AA] genotype; and
- (g) the presence of the homozygous ERCC1 rs11615 [TT] genotype.

6. The use of claim 5, wherein said method comprises assessing if the cancer patient is a responder to treatment with an antibody by the method of any one of claims 1 to 4 and treating the cancer patient with the antibody if the patient has a reduced risk for not being a responder to treatment with the antibody.

7. The use of claim 5 or 6 wherein the treatment regimen comprises a treatment with an antibody-drug conjugate and wherein the antibody is directed against the tumor antigen, the antibody-drug conjugate is preferably an antibody coupled to a radioactive, chemotherapeutic or toxin moiety or wherein the antibody-drug conjugate is an antibody coupled to a cytostatic or cytotoxic compound.
8. The method of any one of claims 1 to 4 or the use of any one of claims 5 to 7 wherein the tumor is a solid tumor.
9. The method of any one of claims 1 to 4 and 8 or the use of any one of claims 5 to 8 wherein the tumor is a gastroesophageal tumor.
10. The method of any one of claims 1 to 4, 8 and 9 or the use of any one of claims 5 to 9 wherein the tumor is an advanced adenocarcinoma of the stomach or the lower esophagus.