CLASSIFYING TUMORS AND PREDICTING RESPONSIVENESS

Background

[0001] Cancer is the second leading cause of death in the United States. Increasingly, immune modulating therapies, such as therapy with immune checkpoint inhibitors (ICI) are being explored as promising potential therapies for many cancers.

Summary

[0001a] In a first aspect of the present disclosure, there is provided a method:

(A) of assessing a tumor's likely responsiveness to immunomodulation therapy, which method comprises
(a) assessing both:
(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and
(ii) status markers of immunomodulatory (IM) status; and
(b) calculating, by means of a computer, an IO score by weighting the subtype markers as likely to indicate non-responsiveness to immunomodulation therapy and the status markers as likely to indicate responsiveness to immunomodulation therapy; or

(B) of assessing a tumor's likely responsiveness to immunomodulation therapy, which method comprises
(a) assessing both:
(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and
(ii) status markers of immunomodulatory (IM) status; and
(b) calculating, by means of a computer, an IO score by weighting the subtype markers as likely to indicate non-responsiveness to immunomodulation therapy and the status markers as likely to indicate responsiveness to immunomodulation therapy;
wherein the subtype markers and status markers are expression levels for a set of genes selected from the group consisting of:

CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10,

COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1., CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1, SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5, TNFAIP8, TNFSF10, RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG, CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273, CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6, KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2, APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1, ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16, GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3, HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R, BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1, C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3, FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1, ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12, MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2, ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1, TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1, ITGBL1, ASPN, PDGFRB, HTRA1, HEG1, ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1, TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19, ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3,

RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, ZCCHC24, and combinations thereof.

[0001b] In another aspect of the present disclosure, there is provided a therapy for use in:

(A) treating cancer, wherein the therapy is an immunomodulation therapy, and wherein: immunomodulation therapy is to be administered to subjects whose tumors have been determined to be responsive to the immunomodulation therapy by assessment of both:

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status;

wherein the subtype markers are considered to indicate likely non-responsiveness to immunomodulation therapy and the status markers are considered to indicate likely responsiveness to immunomodulation therapy;

(B) treating a tumor, wherein:

(a) the tumor is assessed at a first time point by determining both

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status;

wherein the subtype markers are considered to indicate likely non-responsiveness to immunomodulation therapy and the status markers are considered to indicate likely responsiveness to immunomodulation therapy, so that an IO score representing the tumor's likelihood of responding to the immunomodulation therapy is determined;

(b) therapy is selected according to the IO score, wherein the selecting comprises:

(i) initiating or continuing immunomodulation therapy when the IO score meets a threshold determined to correlate with responsiveness to the immunomodulation therapy; and/or

(ii) reducing or withdrawing the immunomodulation therapy and/or initiating or continuing alternative therapy when the IO score meets a threshold determined to correlate with non-responsiveness to the immunomodulation therapy;

(C) treating a subject, wherein the therapy is an immunomodulation therapy, and wherein the therapy is to be administered selectively to subjects who have been assigned a numerical IO score calculated through assessment of each of:

a. Mesenchymal (M) subtype and/or mesenchymal stem-like (MSL) subtype as a negative predictor of responsiveness; and

b. IM status as a positive predictor of responsiveness;

(D) treating cancer, wherein the therapy is ICI therapy, and wherein:

(i) expression levels for one or more genes selected from the group consisting of :

CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1., CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1, SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5, TNFAIP8, TNFSF10, RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG, CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273, CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6, KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2, APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1, ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16, GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3, HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R, BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1, C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2,

ITGAE, STAC3, FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1, ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12, MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2, ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1, TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1, ITGBL1, ASPN, PDGFRB, HTRA1, HEG1, ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1, TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19, ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, and ZCCHC24, is assessed;

(ii) the assessed expression is compared with a set of reference thresholds for the one or more genes; and

(iii) ICI therapy is to be administered to the subject if the comparing determines that the assessed expression levels have a significant pattern relative to their reference thresholds;

(E) treating cancer, wherein the therapy is ICI therapy, and wherein:

(i) expression levels in a sample from a subject suffering from the cancer is assessed for a set of genes selected from the group consisting of :

CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1., CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1, SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5, TNFAIP8, TNFSF10, RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG, CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273, CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6, KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2, APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1, ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16, GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3, HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R, BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1, C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3, FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1, ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12, MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2, ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1, TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1, ITGBL1, ASPN, PDGFRB, HTRA1, HEG1, ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1, TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19, ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14,

KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, ZCCHC24, and combinations thereof,

wherein reference levels for the set have been established, when considered together, to characterize M, IM and MSL character; and

(ii) the assessed expression levels is compared with the set of established reference levels; and; and

(iii) ICI therapy is to be administered to the subject if the comparing determines that the assessed expression levels indicate that the M, IM, and MSL character of the subject's cancer indicate that it is likely to be responsive to the ICI therapy;

(F) treating a subject in whom a biomarker has been detected, wherein the therapy is immunomodulation therapy or therapy, and wherein:
immunomodulation therapy or therapy that sensitizes to immunomodulation therapy if the therapy has been correlated with IM status is to be administered; and
alternative therapy is to be administered if the biomarker has been correlated with M or MSL subtype; or

(G) treating a subject in whom a biomarker has been detected, wherein:
therapy that has been correlated with IM status if the biomarker has also been so correlated is to be administered; and
therapy that has been correlated with M or MSL subtype if the therapy has also been so correlated is to be administered.

[0001c] In yet another aspect of the present disclosure, there is provided a method:

(A) of monitoring therapy administered to a cancer patient, the method comprising steps of:
(a) at each of a plurality of time points, determining both

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status;

wherein the subtype markers are considered to indicate likely non-responsiveness to immunomodulation therapy and the status markers are considered to indicate likely responsiveness to immunomodulation therapy, so that an IO score representing the patient's likelihood of responding to the immunomodulation therapy is determined; and
(b) therapy is to be adjusted in light of a change in the IO score;

(B) comprising steps of:

a. receiving, by a processor of a computing device, data corresponding to levels of a plurality of markers for each of:

    i. a subtype selected from M, MSL, and combinations thereof; and

    ii. an IM status;

b. automatically determining, by the processor, a classification of the subject as non-responsive to a first therapy (e.g. immunomodulation therapy) using the data received in step (a) to produce a numerical score; and, optionally,

c. a second therapy (e.g. an alternative to the first therapy, e.g., an alternative to immunomodulation therapy) is to be prescribed and/or administered to the subject for treatment of the disease, thereby avoiding prescription and/or administration of the first therapy to the subject;

(C) comprising the steps of:

a. receiving, by a processor of a computing device, data corresponding to levels of a plurality of markers for each of:

    i. a subtype selected from M, MSL, and combinations thereof; and

    ii. an IM status;

b. automatically determining, by the processor, a classification of the subject as responsive to a first therapy (e.g. immunomodulation therapy) using the data received in step (a) to produce a numerical score; and, optionally,

c. the first therapy is to be prescribed and/or administered to the subject for treatment of the disease;

(D) of determining a tumor classifier effective to distinguish between responsiveness and non-responsiveness to immunomodulation therapy, the method comprising steps of:

a. Employing elastic net regularized linear models to create individual subclassifying models for a set of subtypes;

b. Training the classifier on a gene expression dataset from a sample of interest; and

c. Assessing the correlation between the classifier and responsiveness to immunomodulation therapy;

(E) of establishing a biomarker indicative of immune microenvironment status, the method comprising steps of:

determining a correlation between a candidate biomarker and one or more of IM status markers and M and MSL subtype markers;

incorporating the candidate biomarker into a complete biomarker that includes both indicators of likely responsiveness and indicators of likely non-responsiveness to immunomodulation therapy;

(F) of establishing a biomarker indicative of immune microenvironment status, the method comprising steps of:
providing a classification system that includes both:

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status; and

has been established to predict responsiveness to immunomodulation therapy by considering both markers that indicate likely non-responsiveness and markers that indicate likely responsiveness to the immunomodulation therapy;

(G) of characterizing a potential cancer therapy by determining that it directly or indirectly correlates with an immunomodulatory (IM) status or with a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL); or

(H) comprising a step of:
detecting in a subject who is a candidate for receiving a particular therapy a biomarker established to correlate with responsiveness or non-responsiveness to the therapy.

[0001d] In a further aspect of the present disclosure, there is provided a therapy for use in treating a subject:

(A) whose tumour has been determined to have an IO score above a threshold established to correlate with responsiveness to the immunomodulation therapy according to the method as disclosed herein, and wherein the therapy is the immunomodulatory therapy according to the method as disclosed herein, optionally wherein:

(i) the immunomodulation therapy is to be selectively administered to subjects whose tumors have been determined to have IO scores above a certain threshold; or

(ii) the immunomodulation therapy is selected from the ICI therapy, CAR-T cell therapy, neoantigen vaccine therapy, or combinations thereof; or

(B) whose tumor has been determined to have an IO score below a certain threshold according to the method as disclosed herein, and wherein the therapy is an alternative therapy to the immunomodulation therapy as disclosed herein, optionally wherein the alternative therapy is kinase inhibitor or other tumor microenvironment modulating therapy.

[0001e] In another aspect of the present disclosure, there is provided an additional therapy for use in treating a subject whose tumour has been determined according to the method as disclosed herein to have an IO score below a threshold established to correlate with responsiveness to the immunomodulation therapy, optionally wherein the additional therapy is selected to target gene pathways associated with negative IO scores, for example wherein:

(a) the immunomodulation therapy is ICI therapy and the additional therapy is not ICI therapy;

(b) the immunomodulation therapy and additional therapy are to be co-administered; or

(c) the immunomodulation therapy and additional therapy are to be administered sequentially.

[0002] The present disclosure provides technologies for determining likelihood of patient responsiveness to certain therapies (e.g., for stratifying patient populations), and for treatment of cancer by administering such therapy to responsive patients and/or populations (and/or withholding such therapy and/or administering alternative therapy to non-responsive patients and/or populations), as defined herein. In particular, the present disclosure provides technologies for determining likelihood of patient responsiveness to immunomodulation therapy.

[0003] Without wishing to be bound by any particular theory, the present disclosure provides an insight that effective biomarkers for responsiveness to relevant therapy (e.g., immunomodulation therapy, and particularly ICI therapy) may be those that capture examples of immunosurveillance, immunosuppression, and immune evasion as a tumor transitions from a proliferative to a metastatic state. Alternatively or additionally, the present disclosure provides an insight that effective biomarkers for responsiveness to immunomodulation therapy may asses one or more features of an immunological state of the tumor microenvironment (TME).

[0004] The present disclosure demonstrates, among other things, that assessment of a mesenchymal (M) gene expression signature, a mesenchymal stem-like (MSL) gene expression signature and an immunomodulatory (IM) gene expression signature can together provide an immuno-oncology score (an IO score) that is an effective biomarker for responsiveness to certain therapies (e.g. immunomodulation therapy, and particularly ICI therapy). In some examples, mesenchymal (M) gene expression signature, mesenchymal stem-like (MSL) gene expression signature and immunomodulatory (IM) gene expression signature are assessed through examination of a set of genes provided herein. In some examples, mesenchymal (M) gene expression signature, mesenchymal stem-like

(MSL) gene expression signature and immunomodulatory (IM) gene expression signature are assessed through examination of genes determined through use of a gene expression algorithm.

[0005] In some examples, the present disclosure provides technologies for monitoring therapy administered to a cancer patient through assessment of an IO score over time. Alternatively or additionally, the present disclosure provides methods of selecting and/or adjusting therapies administered to a cancer patient through assessment of an IO score at multiple time points. In some examples, the present disclosure provides methods for selectively administering one or more therapies to a cancer patient determined to have an IO score meeting a certain threshold value.

[0006] Without wishing to be bound by a particular theory, the present disclosure provides an insight that assessment of an IO score can inform selection of a particular therapy (e.g. immunomodulation therapy, and particularly ICI therapy) for administration to a patient with a malignancy or potential malignancy. In some examples, the present disclosure provides an insight that assessment of an IO score can inform selection of a combination of one or more therapies, either in tandem or in sequence (e.g. comprising one or more immunomodulation therapies).

[0007] The present disclosure demonstrates, among other things, development of a tumor classifier effective to distinguish between responsiveness and non-responsiveness to immunomodulation therapy. In some examples, the present disclosure provides an insight that a tumor classifier can be trained for use in multiple different tumor types.

[0008] Alternatively or additionally, the present disclosure permits assessment of association (*e.g.*, correlation) with classified IM, M, and/or MSL features. In some examples, the present disclosure permits identification and/or characterization of other parameters (*e.g.*, RNA levels, gene expression, gene mutation, protein expression, protein modification, epigenetic modification, *etc.*) for association. In some examples, such associated features may comprise biomarkers that may be detected (*e.g.*, measurement of presence and/or or levels). In some examples, such associated features may comprise a particular form (*e.g.*, variant form (*e.g.*, presence of a particular allele or mutation), modified form (*e.g.*, epigenetic modification of a gene or gene associated sequence, phosphorylation

or glycosylation of a protein, etc.), a particular one of known forms (*e.g.*, splicing forms, allelelic forms, etc.), etc.) of one or more genes or gene products. In some examples, technologies provided herein permit assessment of association with IM, M, and/or MSL features, which can reveal presence and/or development of biological event(s) that recommend particular therapy be used in addition or as an alternative to immunomodulation therapy.

[0009] In some examples, the present disclosure provides a method of characterizing a potential cancer therapy by determining that said therapy directly or indirectly correlates with IM, M, and/or MSL features. In some examples, the present disclosure provides a method comprising a step of detecting in a subject who is a candidate for receiving a particular therapy a biomarker established to correlate with responsiveness or non-responsiveness to the therapy.

[0010] In some examples, the present disclosure provides a method of treating a subject in whom a biomarker has been detected, the method comprising steps of administering immunomodulation therapy or therapy that sensitizes to immunomodulation therapy if the therapy has been correlated with IM status and administering alternative therapy if the biomarker has been correlated with M or MSL subtype.

[0011] In some examples, the present disclosure provides a method of treating a subject in whom a biomarker has been detected, the method comprising steps of administering therapy that has been correlated with IM status if the biomarker has also been so correlated and administering therapy that has been correlated with M or MSL subtype if the therapy has also been so correlated.

[0012] In some examples, mesenchymal (M) gene expression signature, mesenchymal stem-like (MSL) gene expression signature and/or immunomodulatory (IM) gene expression signatures as provided here, and/or models or representations of tumor subtype and/or are used to establish and/or characterize (e.g., validate) biomarkers of tumor subtype or status (i.e., of IM, M, or MSL character), and/or of responsiveness to particular therapy, for example by demonstrating correlation with a provided gene expression signature and/or with a result (e.g., a heat map) of its application to tissue analysis.

[0013] Still further, by demonstrating effectiveness of provided technologies at classifying tumor subtype, status and/or responsiveness, the present disclosure provides technologies that permit investigation and/or interpretation of data such as clinical and/or cell line data, including relevant to development of resistance to one or more particular therapies (e.g., ICI therapy) and/or emergence of additional targets for therapy. Thus, in some examples, the present disclosure provides technologies for identifying and/or characterizing therapeutic targets, for selecting, administering and/or adjusting therapeutic regimens (e.g., to address or anticipate developing resistance and/or emerging target(s) in a particular subject or set of subjects.

[0014] Advantages of certain examples of provided technologies include that such assessment may be of data inputs from any of a variety of platforms; as documented herein, strategies provided by the present disclosure can provide an effective IO score biomarker independent of data input source.

Brief Description of the Drawings

[0015] **Figure 1**: Common Immune Checkpoint Pathways and FDA-Approved ICIs. Figure adapted from Hui *et al.*, "Immune checkpoint inhibitors" *J. Cell Biol.* 218, 2019, incorporated herein by reference in its entirety. Artwork by Neil Smith (nel@neilsmithillustration.co.uk).

[0016] **Figure 2**: Schematic of chimeric antigen receptor (CAR) structure, adapted from Feins et al *et al.*, "An introduction to chimeric antigen receptor (CAR) T-cell immunotherapy for human cancer", *Am J Hematol*. 94, 2019, incorporated herein by reference in its entirety.

[0017] **Figure 3**: Major types of neoantigen vaccines, adapted from Peng *et al.*, "Neoantigen vaccine: an emerging tumor immunotherapy", *Mol. Cancer*, 18, 2019, incorporated herein by reference in its entirety.

[0018] **Figure 4**: Mechanisms of Rescue of CAR T cell Exhaustion with Checkpoint Blockade, adapted from Grosser *et al*., "Combination Immunotherapy with CAR T Cells and

Claims

We claim:

1. A method:

(A) of assessing a tumor's likely responsiveness to immunomodulation therapy, which method comprises

(a) assessing both:

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status; and

(b) calculating, by means of a computer, an IO score by weighting the subtype markers as likely to indicate non-responsiveness to immunomodulation therapy and the status markers as likely to indicate responsiveness to immunomodulation therapy; or

(B) of assessing a tumor's likely responsiveness to immunomodulation therapy, which method comprises

(a) assessing both:

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status; and

(b) calculating, by means of a computer, an IO score by weighting the subtype markers as likely to indicate non-responsiveness to immunomodulation therapy and the status markers as likely to indicate responsiveness to immunomodulation therapy;

wherein the subtype markers and status markers are expression levels for a set of genes selected from the group consisting of:

CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1., CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1, SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5, TNFAIP8, TNFSF10, RARRES3,

APOL3, LINC02446, ZNF683, IFNG, FASLG, CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273, CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6, KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2, APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1, ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16, GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3, HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R, BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1, C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3, FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1, ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12, MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2, ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1, TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1, ITGBL1, ASPN, PDGFRB, HTRA1, HEG1, ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1, TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19, ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, ZCCHC24, and combinations thereof.

2. A therapy for use in:

(A) treating cancer, wherein the therapy is an immunomodulation therapy, and wherein: immunomodulation therapy is to be administered to subjects whose tumors have been determined to be responsive to the immunomodulation therapy by assessment of both:

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status;

wherein the subtype markers are considered to indicate likely non-responsiveness to immunomodulation therapy and the status markers are considered to indicate likely responsiveness to immunomodulation therapy;

(B) treating a tumor, wherein:

(a) the tumor is assessed at a first time point by determining both

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status;

wherein the subtype markers are considered to indicate likely non-responsiveness to immunomodulation therapy and the status markers are considered to indicate likely responsiveness to immunomodulation therapy, so that an IO score representing the tumor's likelihood of responding to the immunomodulation therapy is determined;

(b) therapy is selected according to the IO score, wherein the selecting comprises:

(i) initiating or continuing immunomodulation therapy when the IO score meets a threshold determined to correlate with responsiveness to the immunomodulation therapy; and/or

(ii) reducing or withdrawing the immunomodulation therapy and/or initiating or continuing alternative therapy when the IO score meets a threshold determined to correlate with non-responsiveness to the immunomodulation therapy;

(C) treating a subject, wherein the therapy is an immunomodulation therapy, and wherein the therapy is to be administered selectively to subjects who have been assigned a numerical IO score calculated through assessment of each of:

a. Mesenchymal (M) subtype and/or mesenchymal stem-like (MSL) subtype as a negative predictor of responsiveness; and

b. IM status as a positive predictor of responsiveness;

(D) treating cancer, wherein the therapy is ICI therapy, and wherein:

(i) expression levels for one or more genes selected from the group consisting of :

CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1., CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1, SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5, TNFAIP8, TNFSF10, RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG, CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273, CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6, KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2, APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1, ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16, GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3, HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R, BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1, C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3, FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1, ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12, MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2, ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1, TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1, ITGBL1, ASPN, PDGFRB, HTRA1, HEG1, ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1, TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19, ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20,

CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, and ZCCHC24, is assessed;

(ii) the assessed expression is compared with a set of reference thresholds for the one or more genes; and

(iii) ICI therapy is to be administered to the subject if the comparing determines that the assessed expression levels have a significant pattern relative to their reference thresholds;

(E) treating cancer, wherein the therapy is ICI therapy, and wherein:

(i) expression levels in a sample from a subject suffering from the cancer is assessed for a set of genes selected from the group consisting of :

CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1., CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1, SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5, TNFAIP8, TNFSF10, RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG, CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273, CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6, KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2, APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1, ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16, GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3, HNRNPA1P21,

FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R, BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1, C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3, FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1, ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12, MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2, ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1, TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1, ITGBL1, ASPN, PDGFRB, HTRA1, HEG1, ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1, TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19, ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, ZCCHC24, and combinations thereof, wherein reference levels for the set have been established, when considered together, to characterize M, IM and MSL character; and

(ii) the assessed expression levels is compared with the set of established reference levels; and; and

(iii) ICI therapy is to be administered to the subject if the comparing determines that the assessed expression levels indicate that the M, IM, and MSL character of the subject's cancer indicate that it is likely to be responsive to the ICI therapy;

(F) treating a subject in whom a biomarker has been detected, wherein the therapy is immunomodulation therapy or therapy, and wherein:
immunomodulation therapy or therapy that sensitizes to immunomodulation therapy if the therapy has been correlated with IM status is to be administered; and
alternative therapy is to be administered if the biomarker has been correlated with M or MSL subtype; or
(G) treating a subject in whom a biomarker has been detected, wherein:
therapy that has been correlated with IM status if the biomarker has also been so correlated is to be administered; and
therapy that has been correlated with M or MSL subtype if the therapy has also been so correlated is to be administered.

3. A method:
(A) of monitoring therapy administered to a cancer patient, the method comprising steps of:
(a) at each of a plurality of time points, determining both
(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and
(ii) status markers of immunomodulatory (IM) status;
wherein the subtype markers are considered to indicate likely non-responsiveness to immunomodulation therapy and the status markers are considered to indicate likely responsiveness to immunomodulation therapy, so that an IO score representing the patient's likelihood of responding to the immunomodulation therapy is determined; and
(b) therapy is to be adjusted in light of a change in the IO score;
(B) comprising steps of:

a. receiving, by a processor of a computing device, data corresponding to levels of a plurality of markers for each of:
    i. a subtype selected from M, MSL, and combinations thereof; and
    ii. an IM status;
b. automatically determining, by the processor, a classification of the subject as non-responsive to a first therapy (e.g. immunomodulation therapy) using the data received in step (a) to produce a numerical score; and, optionally,

c. a second therapy (e.g. an alternative to the first therapy, e.g., an alternative to immunomodulation therapy) is to be prescribed and/or administered to the subject for treatment of the disease, thereby avoiding prescription and/or administration of the first therapy to the subject;

(C) comprising the steps of:

a. receiving, by a processor of a computing device, data corresponding to levels of a plurality of markers for each of:
    i. a subtype selected from M, MSL, and combinations thereof; and
    ii. an IM status;
b. automatically determining, by the processor, a classification of the subject as responsive to a first therapy (e.g. immunomodulation therapy) using the data received in step (a) to produce a numerical score; and, optionally,
c. the first therapy is to be prescribed and/or administered to the subject for treatment of the disease;

(D) of determining a tumor classifier effective to distinguish between responsiveness and non-responsiveness to immunomodulation therapy, the method comprising steps of:

a. Employing elastic net regularized linear models to create individual subclassifying models for a set of subtypes;
b. Training the classifier on a gene expression dataset from a sample of interest; and
c. Assessing the correlation between the classifier and responsiveness to immunomodulation therapy;

(E) of establishing a biomarker indicative of immune microenvironment status, the method comprising steps of:

determining a correlation between a candidate biomarker and one or more of IM status markers and M and MSL subtype markers;

incorporating the candidate biomarker into a complete biomarker that includes both indicators of likely responsiveness and indicators of likely non-responsiveness to immunomodulation therapy;

(F) of establishing a biomarker indicative of immune microenvironment status, the method comprising steps of:

providing a classification system that includes both:

(i) subtype markers of a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL), and combinations thereof; and

(ii) status markers of immunomodulatory (IM) status; and

has been established to predict responsiveness to immunomodulation therapy by considering both markers that indicate likely non-responsiveness and markers that indicate likely responsiveness to the immunomodulation therapy;

(G) of characterizing a potential cancer therapy by determining that it directly or indirectly correlates with an immunomodulatory (IM) status or with a subtype selected from mesenchymal (M), mesenchymal stem-like (MSL); or

(H) comprising a step of:

detecting in a subject who is a candidate for receiving a particular therapy a biomarker established to correlate with responsiveness or non-responsiveness to the therapy.

4. The method of claim 1(A), further comprising:

(a) a step of identifying a subject whom the immunomodulation therapy is to be administered, wherein the tumor of the subject has been determined to have an IO score above a threshold established to correlate with responsiveness to the immunomodulation therapy, optionally wherein:

(i) the immunomodulation therapy is to be selectively administered to subjects whose tumors have been determined to have IO scores above a certain threshold; or

(ii) the immunomodulation therapy is selected from the ICI therapy, CAR-T cell therapy, neoantigen vaccine therapy, or combinations thereof; or

(b) a step of identifying a subject whom an alternative therapy is to be administered, wherein the tumor of the subject has been determined to have an IO score below a certain threshold, optionally wherein the alternative therapy is kinase inhibitor or other tumor microenvironment modulating therapy.

5. A therapy for use in treating a subject:

(A) whose tumour has been determined to have an IO score above a threshold established to correlate with responsiveness to the immunomodulation therapy according to the method of

claim 1(A), and wherein the therapy is the immunomodulatory therapy according to the method of claim 1(A), optionally wherein:

(i) the immunomodulation therapy is to be selectively administered to subjects whose tumors have been determined to have IO scores above a certain threshold; or

(ii) the immunomodulation therapy is selected from the ICI therapy, CAR-T cell therapy, neoantigen vaccine therapy, or combinations thereof; or

(B) whose tumor has been determined to have an IO score below a certain threshold according to the method of claim 1(A), and wherein the therapy is an alternative therapy to the immunomodulation therapy according to claim 1(A), optionally wherein the alternative therapy is kinase inhibitor or other tumor microenvironment modulating therapy.

6. The method of claim 3(A), wherein:

(a) an increase in IO score, or an IO score greater than a predefined threshold, indicates an increased likelihood of responding to the immunomodulation therapy; or

(b) a decrease in IO score, or an IO score less than a predefined threshold, indicates a reduced likelihood of responding to the immunomodulation therapy.

7. The therapy for use according to claim 2(B), wherein:

(a) the immunomodulation therapy is selected from the ICI therapy, CAR-T cell therapy, neoantigen vaccine therapy, or combinations thereof; or

(b) the alternative therapy is kinase inhibitor therapy.

8. The therapy for use according to claim 2(C), wherein the assigned IO score is above a threshold established to distinguish between responsive and non-responsive historical subjects who have received the immunomodulation therapy.

9. The method of claim 3(D), wherein:

(a) the classifier comprises a set of between 75 and 100 genes;

(b) the classifier comprises a set of between 50 and 75 genes;

(c) the classifier comprises a set of between 25 and 50 genes;

(d) the classifier comprises a set of less than 25 genes; or

(e) the subtypes are defined based upon previously established models, optionally wherein the classifier comprises a reduced gene set compared to previously established models.

10. The method of claim 1(B), wherein the subtype markers and status markers comprise at least one gene from one or more gene groups below:

Group A: CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1;

Group B1: CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10;

Group B2: COL2A1, FOXC1, KRT16, MIA, SFRP1;

Group B3: APOD, ASPN, HTRA1;

Group C1: SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5;

Group C2: TNFAIP8, TNFSF10;

Group C3: RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG;

Group C4: CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273;

Group C5: CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6;

Group C6: KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2;

Group C7: APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1;

Group C8: ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16;

Group C9: GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3;

Group C10: HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R;

Group C11: BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1;

Group C12: C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3;

Group C13: FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1;

Group C14: ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12;

Group C15: MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2;

Group C16: ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1;

Group C17: TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1;

Group C18: ITGBL1, ASPN, PDGFRB, HTRA1, HEG1;

Group C19: ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1;

Group C20: TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19; and

Group D1: ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, ZCCHC24, optionally wherein:

(a) the subtype markers and status markers comprise at least one gene from five or more of the gene groups, optionally

wherein the subtype markers and status markers comprise at least one gene from ten or more of the gene groups, for example

wherein the subtype markers and status markers comprise at least one gene each of the gene groups; or

(b) the subtype markers and status markers comprise:

(i) at least one gene selected from Group A;

(ii) at least one gene selected from any one of Group B1, Group B2, or Group B3;

(iii) at least one gene selected from any one of Group C1, Group C2, Group C3, Group C4, Group C5, Group C6, Group C7, Group C8, Group C9, Group C10, Group C11, Group C12, Group C13, Group C14, Group C15, Group C16, Group C17, Group C18, Group C19, Group C20; and

(iv) at least one gene selected from Group D1.

11. The method of claim 1(A), further comprising a step of identifying a subject whose tumor has been determined to have an IO score below a certain threshold and whom an additional therapy is to be administered, optionally wherein the additional therapy is selected to target gene pathways associated with negative IO scores, for example wherein:

(a) the immunomodulation therapy is ICI therapy and the additional therapy is not ICI therapy;

(b) the immunomodulation therapy and additional therapy are to be co-administered; or

(c) the immunomodulation therapy and additional therapy are to be administered sequentially.

12. The method of claim 4(b) or therapy for use according to claim 5, wherein the alternative therapy is selected to target gene pathways associated with negative IO scores, optionally:

(a) wherein the immunomodulation therapy is ICI therapy and the alternative therapy is not ICI therapy; or

(b) wherein:

(i) the alternative therapy is to be administered; and

(ii) the IO score is determined after alternative therapy administration;

wherein, if the IO score has changed to be above a certain threshold, the alternative therapy is either:

to be discontinued in favor of immunomodulation therapy; or

to be continued along with co-administration of immunomodulation therapy.

13. An additional therapy for use in treating a subject whose tumour has been determined according to the method of claim 1(A) to have an IO score below a threshold established to correlate with responsiveness to the immunomodulation therapy, optionally wherein the additional therapy is selected to target gene pathways associated with negative IO scores, for example wherein:

(a) the immunomodulation therapy is ICI therapy and the additional therapy is not ICI therapy;

(b) the immunomodulation therapy and additional therapy are to be co-administered; or

(c) the immunomodulation therapy and additional therapy are to be administered sequentially.

14. The method of claim 3(E), wherein:

(a) the IM status markers and the M and MSL subtype markers comprise at least one gene from one or more gene groups below:

Group A: CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1;

Group B1: CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10;

Group B2: COL2A1, FOXC1, KRT16, MIA, SFRP1;

Group B3: APOD, ASPN, HTRA1;

Group C1: SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5;

Group C2: TNFAIP8, TNFSF10;

Group C3: RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG;

Group C4: CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273;

Group C5: CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6;

Group C6: KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2;

Group C7: APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4, CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1;

Group C8: ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16;

Group C9: GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3;

Group C10: HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R;

Group C11: BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1;

Group C12: C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3;

Group C13: FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1;

Group C14: ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12;

Group C15: MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2;

Group C16: ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1;

Group C17: TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1;

Group C18: ITGBL1, ASPN, PDGFRB, HTRA1, HEG1;

Group C19: ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1;

Group C20: TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19; and

Group D1: ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, ZCCHC24;

(b) the IM status markers and the M and MSL subtype markers are identified by a gene expression algorithm; or

(c) the biomarker comprises one or more gene variants, optionally

wherein the one or more gene variants may present differences in gene expression.

15. The method of claim 3(F), wherein:

(a) the markers are or comprise expression levels for a set of genes selected from the group consisting of:

CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1., CCL5, CD52, CXCL11, CXCL13, DUSP5, GZMB, IDO1, IL23A, ITM2A, KMO, KYNU, PSMB9, PTGDS, RARRES3, RTP4, S100A8, SPTLC2, TNFAIP8, TNFSF10, COL2A1, FOXC1, KRT16, MIA, SFRP1, APOD, ASPN, HTRA1, SAMSN1, CD80, CLEC7A, PDCD1LG2, CD274, S100A8, KYNU, LINC02195, IL9R, DUSP5, TNFAIP8, TNFSF10, RARRES3, APOL3, LINC02446, ZNF683, IFNG, FASLG, CD48, CD52, C16orf54, TESPA1, JAML, GMFG, ARHGAP15, TMEM273, CD3G, TIGIT, SIRPG, TRAC, CD3E, CD2, TRBV28, CD3D, TRBC2, CCR5, CD8A, CCL5, IL2RB, CXCR6, KMO, SNX10, PIK3AP1, SLC7A7, VCAM1, RASSF4, TFEC, HAVCR2, APOL6, IDO1, CXCL9, GBP5, GBP1, GBP4,

CXCL11, CXCL10, LAP3, STAT1, WARS1, SAMHD1, ZBP1, OASL, EPSTI1, IL15RA, USP30-AS1, BATF2, ETV7, PSMB10, RTP4, CARD16, GZMB, GZMH, GNLY, CD8B, CTSW, CST7, NKG7, GZMA, PRF1, CD247, SLA2, PDCD1, CD7, LAG3, HNRNPA1P21, FOXP3, CCR8, CXCL13, AIM2, IL2RA, ICOS, CTLA4, TNFRSF9, IL21R, BTN3A3, BTN3A1, TAP2, NLRC5, HLA-F, PSMB8, PSMB9, TAP1, HCP5, UBE2L6, PSME2, IRF1, C19orf38, IGFLR1, LINC01943, RAB33A, SLC2A6, IFI30, LILRB3, IL23A, PSME2P2, ITGAE, STAC3, FOXC1, ADAMTS9-AS2, RGN, KL, ADAMTS9-AS1, WDFY3-AS2, PTH1R, PLEKHH2, WSCD1, CABP1, CEP112, TMEM47, RCAN2, LIN7A, LEPR, PDGFA, SERTAD4-AS1, ADH1B, C7, CCL14, SELP, ACKR1, MMRN1, ITM2A, AQP1, ABI3BP, P2RY12, MPRIP, KIF13B, FYCO1, SPTLC2, ADGRA3, RBFOX2, ITGB4, KRT17, KRT16, KRT14, KRT5, DSG3, COL17A1, TMEM119, PODN, SVEP1, LAMA2, COL14A1, FGF7, OGN, PRELP, ELN, MFAP4, SSC5D, PTGDS, CHRDL1, ITGBL1, ASPN, PDGFRB, HTRA1, HEG1, ZCCHC24, SGCD, SRPX, APOD, SHC4, MIA, IL17D, LRRN4CL, BOC, PDZRN3, SFRP1, TCF7L1, CACNA1G, SPEG, COL2A1, CRISPLD1, PIANP, NACAD, EFNB3, PCYT1B, RGMA, GLI2, PCDH19, ABCA8, ADRA2A, AKAP12, ALDH3B2, APOD, ART3, ASPN, AZGP1, BLVRB, C7, CCL5, CD36, CD52, CDC20, CHI3L1, COL2A1, COL5A1, COL5A2, CRAT, CROT, CXCL10, CXCL11, CXCL13, CYP4F8, DBI, DEFB1, DHCR24, DUSP5, FABP7, FASN, FGFR4, FGL2, FOXA1, FOXC1, GABRP, GALNT7, GBP1, GCHFR, GPR87, GZMB, HGD, HTRA1, IDO1, IGFBP4, IGHM, IGJ, IL23A, IL33, INPP4B, ITM2A, JAM2, KCNK5, KIAA1324, KMO, KRT14, KRT16, KRT17, KRT6A, KRT6B, KYNU, LBP, LHFP, IGKC, MFAP4, MIA, MID1, MYBL1, NEK2, NTN3, OGN, PI3, PLEKHB1, PMAIP1, PSMB9, PTGDS, RARRES3, RTP4, S100A1, S100A7, S100A8, SCRG1, SEMA3C, SERHL2, SFRP1, SIDT1, SOX10, SPDEF, SPRR1B, SPTLC2, SRPX, TCF7L1, TFAP2B, THBS4, TNFAIP8, TNFSF10, TRIM68, TSC22D3, UBD, UGT2B28, XBP1, ZCCHC24, and combinations thereof; or
(b) the markers are or indicate, presence or level of a particular form of one or more genes or gene products.

16. The method of claim 15, wherein the candidate biomarker is selected from the group consisting of presence and level of a particular form of a gene or gene product, for example wherein:

(a) the candidate biomarker is or comprises presence or level of one or more miRNA species;
(b) the candidate biomarker is or comprises presence or level of one or more epigenetic modifications;
(c) the candidate biomarker is or comprises presence or level of one or more gene mutations;
(d) the candidate biomarker is or comprises presence or level of one or more gene transcript forms; or
(e) the candidate biomarker is or comprises presence or level of one or more proteins or forms thereof.