target these wild-type biomarkers and be specific to mutations as well. Of all cancers, lung cancers are mostly affected due to the sheer number of mutations that vary from patient to patient. Within lung cancer, non-small cell lung cancer (NSCLC), accounts for almost 80% of the cases (Inoue et al., Cancer Chemother Pharmacol 2015; 76: 155-161) and almost 10% of NSCLC patients harbor EGFR mutations. In addition to EGFR, other dominant mutations such as KRAS or ALK are also prevalent. Hence, efforts have been made to slowly bring personalized medicine to target the specific mutations. Of most drugs, tyrosine kinase inhibitors (TKI) are used to target these mutations. Primarily the first line and second line treatments in lung cancer cases include FDA approved cocktail of immunotherapy PD-L1 checkpoint inhibitors with TKIs such as erlotinib or gefitinib. Both these small molecule inhibitors bind to the intracellular domain of EGFR and specifically inhibits its downstream pathway. Additionally, these drugs can bind and inhibit other tyrosine kinase receptors as well albeit with low specificity. Xu Y et al., Cancer Biol Ther 2010; 9: 572-582. Both drugs target mutated EGFR that is very common in NSCLC patients. In recent years, there is a dramatic decrease in overall survival rate due to an increase in cases with treatment relapse. Much of the relapse has been tied to acquired drug resistance. Due to resistance, the drug action often fails before the end of the treatment duration. Therefore, several studies are now underway to recognize the cause of resistance in hopes to increase the drug efficacy. However, the underlying causes are still not well understood.

SUMMARY

[0004] The present disclosure is drawn to methods of sensitizing and/or resensitizing treatment-resistant cancer cells to cancer treatment. In certain examples, the method comprises reducing the expression of at least two upregulated biomarker genes of the cancer cell. In certain examples, the cancer treatment can be administration of an anticancer drug. In certain examples, expression of at least one or expression of at least two of the upregulated cancer biomarker genes is reduced by the administration of an inhibitor. In certain examples, the inhibitor is a small molecule inhibitor, an inhibitory antibody, and/or an inhibitory nucleic acid. In certain examples, the nucleic acid can be an inhibitory siRNA. In certain examples, two of the at least two upregulated cancer biomarkers genes are AXL and FN14.

[0004a] In one aspect, the present disclosure refers to an *in vitro* or *ex vivo* method of sensitizing a treatment-resistant cancer cell to a cancer treatment, the method comprising reducing the expression of at least two upregulated biomarker genes of the cancer cell, optionally wherein the cancer treatment is administration of an anticancer drug.

[0004b] In another aspect, the present disclosure refers to use of an inhibitor in the manufacture of a medicament for sensitizing a treatment-resistant cancer cell to a cancer treatment, wherein the inhibitor is to be administered to reduce the expression of at least two upregulated biomarker genes of the cancer cell; optionally wherein the cancer treatment is administration of an anticancer drug.

[0005] In any examples of the methods disclosed herein, the expression of the upregulated cancer biomarker genes can be reduced by nanoparticle delivery of inhibitory siRNAs attached to a nanoparticle. In certain examples, the nanoparticle can be targeted to the cancer cell via an

antibody or antigen-binding portion thereof specific to a cell-surface marker of the cancer cell. In certain examples, the nanoparticle can be taken into the cell by receptor mediated endocytosis. In certain examples, the cell-surface marker is selected from the group consisting of EGFR, cMET, HER2, HGRF, and PD-L1.

[0006] In any examples of the methods disclosed herein, the method further comprises administering the cancer treatment to which the treatment-resistant cancer is resistant. In certain examples, the treatment-resistant cancer is resistant to is a tyrosine kinase inhibitor. In certain examples, the treatment-resistant cancer is resistant to the anitcancer drug Erlotinib, Cisplatin, Gefitinib, or Dasatinib. In certain examples, the drug can be delivered by the nanoparticle delivering at least one of the inhibitors. In certain examples, the cancer can be non-small cell lung cancer (NSCLC), pancreatic cancer, breast cancer, or colorectal cancer.

[0007] The present disclosure also provides for methods of treating and/or preventing a cancer. In certain examples, the method comprises administering, to a subject having a cancer and/or tumor: (i) inhibitors of at least two upregulated biomarker genes of the cancer and/or tumor and (ii) a cancer treatment. In certain examples, the administration kills the cancer and/or delays the growth of and/or reduces the size of the tumor. In certain examples, the growth of the tumor can be inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor can be decreased by at least about 25%, 30%, 40%, or 50%, in comparison to an untreated control. In certain examples, the growth of the tumor can be inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor can be decreased by at least about 25%, 30%, 40%, or 50%, in comparison to a control treated with only one of the inhibitors and the cancer treatment. In certain examples, the growth of the tumor can be inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor can be decreased by at least about 25%, 30%, 40%, or 50%, in comparison to a control treated with the cancer treatment alone. In certain examples, the size of the tumor can be reduced by at least about 25%, 30%, 40%, or 50%.

[0007a] In another aspect, the present disclosure refers to use of inhibitors of at least two upregulated biomarker genes of the cancer and/or tumor in the manufacture of a medicament for treating and/or preventing a cancer, wherein the medicament is to be administered with a cancer treatment to a subject having a cancer and/or tumor, wherein upon administration the medicament and the cancer treatment kill the cancer and/or delay the growth of and/or reduces the size of the tumor, optionally wherein: (i) the growth of the tumor is inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor is decreased by at least about 25%, 30%, 40%, or 50%, in comparison to an untreated control; (ii) the growth of the tumor is inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor is decreased by at least about 25%, 30%, 40%, or 50%, in comparison to a control treated with only one of the inhibitors and the cancer treatment; or (iii) the growth of the tumor is inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor is decreased by at least about 25%, 30%, 40%, or 50%, in comparison to a control treated with the cancer treatment alone.

[0008] In any examples of the methods disclosed herein, the death of the cancer and/or inhibition or delay of growth and/or the decrease in tumor size resulting from administration of the at least two inhibitors together, in conjunction with the cancer treatment, can be greater than the additive effect on cancer death and/or growth and/or tumor size resulting from administration of the inhibitors separately in conjunction with the cancer treatment. In other words, the method produces a synergistic effect.

[0009] In any examples of the methods disclosed herein, the cancer treatment can be an anticancer drug. In any examples of the methods disclosed herein, two of the at least two upregulated cancer biomarker genes can be AXL and FN14. In any examples of the methods disclosed herein, the inhibitors can be administered by nanoparticle delivery of inhibitory siRNA attached to the surface of the nanoparticle. In certain examples, the nanoparticle can be targeted to the tumor via an antibody or antigen-binding portion thereof specific to a cell-surface marker of a tumor cell. In certain examples, the anticancer drug can be administered by nanoparticle delivery. In certain examples, the same nanoparticle delivers at least one of the inhibitory siRNAs. In any examples of the methods disclosed herein, the anticancer drug can be a tyrosine kinase inhibitor. In certain examples, the anticancer drug can be Erlotinib, Cisplatin, Gefitinib, or Dasatinib.In any examples of the methods disclosed herein, the cancer can be non-small cell lung cancer (NSCLC), pancreatic cancer, breast cancer, or colorectal cancer. In any examples of the methods disclosed herein, administration of any of the inhibitors, nanoparticles, and/or anticancer drug can be intravenous.

[0010] The present disclosure also provides for compositions comprising a nanoparticle and at least one or at least two siRNAs capable of inhibiting the expression of one or more overexpressed cancer biomarker genes. In certain examples, the composition is lyophilized. In certain examples, the siRNAs are attached to the surface of the nanoparticle. In certain examples, the attachment of the siRNA to the surface of the nanoparticle inhibits interaction of the siRNA with nuclease proteins and/or inhibits degradation of the siRNA. In certain examples, this inhibition of interaction of the siRNA with nuclease proteins and/or inhibition of degradation of the siRNA can occur in the bloodstream of a subject.

[0010a] In another aspect, the present disclosure refers to a composition comprising a nanoparticle and at least one or at least two siRNAs capable of inhibiting the expression of one or more overexpressed cancer biomarker genes, optionally wherein the composition is lyophilized.

[0011] In any examples of the compositions disclosed herein, the upregulated biomarker genes can be selected from the group consisting of EGRF, KRAS, HER-2, AXL, and FN14. In certain apsects, the upregulated biomarker genes can be optionally selected from the group consisting of AXL, Her-2, and FN14. In any examples of the compositions disclosed herein, the composition can further comprise an anticancer drug. In certain examples, the anticancer drug can be selected from the group consisting of Erlotinib, Cisplatin, Gefitinib, and Dasatinib.

[0012] In any examples of the compositions disclosed herein, the composition can be targeted to a cancer cell via an antibody or antigen-binding portion thereof portion thereof specific to a cell-surface marker of the cancer cell that is attached to the nanoparticle. In certain examples, the cell-surface marker can facilitate receptor-mediated endocytosis. In any examples of

the compositions disclosed herein, the anticancer drug can be contained within the nanoparticle. In certain examples, the nanoparticle can degrade within a cell to release the drug.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0013] **Figure 1.** **Figure 1** shows activation of RISC complex using siRNA to degrade AXL mRNA in the cytoplasm.

[0014] **Figure 2.** **Figure 2** shows the formation of indel in the AXL gene using the complex formed by CRISPR RNA, Tracer guide RNA, and the Cas9 endonuclease protein.

[0015] **Figure 3.** **Figure 3** shows the three components needed to target, release drug and destroy resistance causing oncogenic mRNA within cell cytoplasm.

[0016] **Figure 4.** **Figure 4** shows the design of an antibody conjugated porous drug encapsulated multifunctional nanoparticle with siRNA on its surface. These particles, once internalized, will degrade within the cytoplasm using proteases and release both drug and siRNA to block and inhibit EGFR pathway and AXL pathway respectively.

[0017] **Figure 5A,B.** **Figure 5A and 5B** show standard curves for (A) Ab-Cy5 using Cetuximab and (B) siAXL-Cy5.

[0018] **Figure 6A-C.** **Figure 6A, 6B, and 6C** show TEM images for (A) Gelatin nanoparticle (G) (B) antibody conjugated GAb and (C) antibody and siRNA conjugated GAbsiAXL.

[0019] **Figure 7A.** **Figure 7A** shows the stability of Gelatin nanoparticle constructs evaluated through change in surface potential over time.

[0020] **Figure 7B.** **Figure 7B** shows the stability of Gelatin nanoparticle constructs evaluated through change in hydrodynamic size over time in (panel (i)) water, (panel (ii)) 1X PBS, (panel (iii)) RPMI media. Averaged results are shown in (panel (iv)).

[0021] **Figure 8A,B.** **Figure 8A and 8B** show the internalization of targeted constructs in (A) H820 and (B) HCC827 cells (40x). Results indicate gelatin particles (Fluorescein) are only internalized when antibody is present on surface. Similarly, siRNA is internalized when gelatin particles are internalized by antibody targeting.

[0022] **Figure 9A,B.** **Figure 9A and 9B** show the internalization of targeted constructs in HCC827 cells: (A) GAb (B) GabsiAXL-Cy5 (60x). Results indicate presence of high quantities of siRNA (Cy5) in the cytoplasm of cells after internalization of construct.

CLAIMS

What is claimed is:

1. An *in vitro* or *ex vivo* method of sensitizing a treatment-resistant cancer cell to a cancer treatment, the method comprising reducing the expression of at least two upregulated biomarker genes of the cancer cell, optionally wherein the cancer treatment is administration of an anticancer drug.

2. The method of claim 1, wherein expression of at least one or expression of at least two of the upregulated biomarker genes is reduced by the administration of an inhibitor.

3. Use of an inhibitor in the manufacture of a medicament for sensitizing a treatment-resistant cancer cell to a cancer treatment, wherein the inhibitor is to be administered to reduce the expression of at least two upregulated biomarker genes of the cancer cell; optionally wherein the cancer treatment is administration of an anticancer drug.

4. The method of claim 2 or the use of claim 3, wherein the inhibitor is a small molecule inhibitor, an inhibitory antibody, or an inhibitory nucleic acid, optionally wherein the nucleic acid is an inhibitory siRNA.

5. The method of any one of claims 1, 2 and 4 or the use of claim 3 or 4, wherein two of the at least two upregulated cancer biomarkers genes are AXL and FN14.

6. The method of any one of claims 2, 4 and 5 or the use of any one of claims 3-5, wherein the expression of the upregulated cancer biomarker genes is reduced by nanoparticle delivery of inhibitory siRNAs attached to a nanoparticle, optionally wherein the nanoparticle is targeted to the cancer cell via an antibody or antigen-binding portion thereof specific to a cell-surface

marker of the cancer cell, optionally wherein the nanoparticle is taken into the cell by receptor mediated endocytosis, and optionally wherein the cell-surface marker is selected from the group consisting of EGFR, cMET, HER2, HGRF, and PD-L1.

7. The method of any one of claims 1, 2 and 4-6 or the use of any one of claims 3-6, wherein the cancer treatment to which the treatment-resistant cancer is resistant is to be administered.

8. The method of any one of claims 1, 2 and 4-7 or the use of any one of claims 3-7, wherein the treatment-resistant cancer is resistant to is a tyrosine kinase inhibitor.

9. The method of any one of claims 1, 2 and 4-8 or the use of any one of claims 3-8, wherein the treatment-resistant cancer is resistant to the anitcancer drug Erlotinib, Cisplatin, Gefitinib, or Dasatinib, optionally wherein the drug is to be delivered by the nanoparticle delivering at least one of the inhibitors.

10. The method of any one of claims 1, 2 and 4-9 or the use of any one of claims 3-9, wherein the cancer is non-small cell lung cancer (NSCLC), pancreatic cancer, breast cancer, or colorectal cancer.

11. Use of inhibitors of at least two upregulated biomarker genes of the cancer and/or tumor in the manufacture of a medicament for treating and/or preventing a cancer, wherein the medicament is to be administered with a cancer treatment to a subject having a cancer and/or tumor, wherein upon administration the medicament and the cancer treatment kill the cancer and/or delay the growth of and/or reduces the size of the tumor, optionally wherein: (i) the

growth of the tumor is inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor is decreased by at least about 25%, 30%, 40%, or 50%, in comparison to an untreated control; (ii) the growth of the tumor is inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor is decreased by at least about 25%, 30%, 40%, or 50%, in comparison to a control treated with only one of the inhibitors and the cancer treatment; or (iii) the growth of the tumor is inhibited or delayed by at least about 25%, 30%, 40%, or 50% and/or the size of the tumor is decreased by at least about 25%, 30%, 40%, or 50%, in comparison to a control treated with the cancer treatment alone.

12. The use of claim 11, wherein the size of the tumor is reduced by at least about 25%, 30%, 40%, or 50%.

13. The use of claim 11 or 12, wherein the death of the cancer and/or inhibition or delay of growth and/or the decrease in tumor size resulting from administration of the at least two inhibitors together, in conjunction with the cancer treatment, is greater than the additive effect on cancer death and/or growth and/or tumor size resulting from administration of the inhibitors separately in conjunction with the cancer treatment.

14. The use of any one of claims 11 to 13, wherein the cancer treatment is an anticancer drug.

15. The use of any one of claims 11 to 14, wherein two of the at least two upregulated cancer biomarker genes are AXL and FN14.

16. The use of any one of claims 11 to 15, wherein the inhibitors are to be administered by nanoparticle delivery of inhibitory siRNA attached to the surface of the nanoparticle, optionally wherein the nanoparticle is targeted to the tumor via an antibody or antigen-binding portion thereof specific to a cell-surface marker of a tumor cell.

17. The use of any one of claims 14 to 16, wherein the anticancer drug is to be administered by nanoparticle delivery, optionally with the same nanoparticle delivering at least one of the inhibitory siRNAs.

18. The use of any one of claims 14 to 17, wherein the anticancer drug is a tyrosine kinase inhibitor, optionally wherein the anticancer drug is Erlotinib, Cisplatin, Gefitinib, or Dasatinib.

19. The use of any one of claims 11 to 18, wherein the cancer is non-small cell lung cancer (NSCLC), pancreatic cancer, breast cancer, or colorectal cancer.

20. The use of any one of 14 to 19, wherein administration of any of the inhibitors, nanoparticles, and/or anticancer drug is intravenous.

21. A composition comprising a nanoparticle and at least one or at least two siRNAs capable of inhibiting the expression of one or more overexpressed cancer biomarker genes, optionally wherein the composition is lyophilized.

22. The composition of claim 21, wherein the siRNAs are attached to the surface of the nanoparticle.

23. The composition of claim 22, wherein the attachment of the siRNA to the surface of the nanoparticle inhibits interaction of the siRNA with nuclease proteins and/or inhibits degradation of the siRNA, optionally in the bloodstream of a subject.

24. The composition of any one of claims 21 to 23, wherein the upregulated biomarker genes are selected from the group consisting of EGRF, KRAS, HER-2, AXL, and FN14, optionally selected from the group consisting of AXL, Her-2, and FN14.

25. The composition of any one of claims 21 to 24, wherein the composition further comprises an anticancer drug, optionally wherein the anticancer drug is selected from the group consisting of Erlotinib, Cisplatin, Gefitinib, and Dasatinib.

26. The composition of any one of claims 21 to 25, wherein the composition is targeted to a cancer cell via an antibody or antigen-binding portion thereof portion thereof specific to a cell-surface marker of the cancer cell that is attached to the nanoparticle, optionally wherein the cell-surface maker facilitates receptor-mediated endocytosis.

27. The composition of any one of claims 21 to 26, wherein the anticancer drug is contained within the nanoparticle, optionally wherein the nanoparticle degrades within a cell to release the drug.