

CLAIMS

- 1) Method for the *in vitro* diagnosis of head and neck cancer, said method comprising detecting, in a biological sample, a mutation in genes consisting of TP53, CDKN2A and FAT1 genes, wherein the presence of at least one mutation in one, two or all of said genes in comparison to the respective wild type sequence indicates the presence of head and neck cancer.
- 2) Method according to claim 1, wherein the method comprises detecting a mutation only in TP53, CDKN2A and FAT1 genes and, optionally, also in PIK3CA gene, whereas the method does not comprise detecting a mutation or other aberrations in other genes.
- 3) Method according to anyone of claims 1-2, wherein the presence of a total of at least two mutations in said one, two or all of said genes in comparison to the respective wild type sequence indicates the presence of:
- head and neck cancer which is a immunotherapy responsive head and neck cancer, and/or
- head and neck cancer in advanced stage,
- wherein said at least two mutations are present in the same gene or in different genes among said one, two or all of said genes.
- 4) Method according to anyone of claims 1-3, wherein detecting a mutation is carried out on the entire coding regions of TP53, CDKN2A and FAT1 genes.
- 5) Method according to anyone of claims 1-4, wherein said biological sample is chosen from the group consisting of a liquid biological sample, such as a liquid biopsy, or a tissue sample, wherein said biological sample is fresh, frozen or formalin-fixed paraffin-embedded.
- 6) Method according to claim 5, wherein said liquid biopsy is chosen from the group consisting of plasma sample or saliva sample, whereas said tissue sample is chosen from the group consisting of a tumor sample, lymph node sample or a resection margin sample.
- 7) Method according to anyone of claims 1-6, wherein said method is carried out on both a liquid sample and a tissue sample.

8) Method according to anyone of claims 1-7, said method comprising the following steps:

a) extracting DNA from a biological sample;

b) sequencing all of TP53, CDKN2A and FAT1 genes; and

5 c) detecting mutations in said genes by comparing each sequence of said genes obtained in step b) with the respective wild type sequence.

9) Method according to any one of claims 1-7, wherein the method is carried out by Next Generation Sequencing technologies.

10) Method according to any one of claims 1-9, said method further comprising detecting the following mutations in PIK3CA gene, said mutations having the following COSMIC_id numbers:

12458

12459

12461

15 12463

12464

12580

12582

12584

20 12590

12591

12592

12597

13570

25 13594

17442

17444

17445

17449

30 21451

21462

22540

24712

	24714
	249872
	249908
	25041
5	25085
	25086
	27133
	27134
	27155
10	27156
	27158
	27273
	27374
	27502
15	27505
	28938
	29110
	29313
	36285
20	36286
	36289
	6147
	746
	754
25	757
	759
	760
	762
	763
30	764
	765
	766
	767

	769
	770
	771
	772
5	773
	774
	775
	776
	777
10	778
	94984
	125368
	12579
	12589
15	14052
	163484
	166154
	21448
	230020
20	24710
	24711
	27490
	27497
	27499
25	39166
	41785
	51258
	51259
	6145
30	6149
	6150
	749
	750

758
 86045
 86949
 86950
 5 87210
 125370
 94985
 94986
 94987
 10 COSM87306

11) Kit for the *in vitro* diagnosis of head and neck cancer, said kit comprising suitable reagents for sequencing all TP53, CDKN2A and FAT1 genes and detecting mutations in said genes in comparison to the respective wild type sequence.

15 12) Kit according to claim 11, wherein the kit comprises reagents for sequencing only TP53, CDKN2A and FAT1 genes and, optionally, also PIK3CA gene, whereas the kit does not comprise any reagent for sequencing other genes.

20 13) Kit according to anyone of claims 11-12, wherein said suitable reagents for sequencing TP53, CDKN2A and FAT1 genes comprise primers for the amplification of said genes, wherein

for the amplification of FAT1 gene, each primer pair has respectively the following initial position of forward primer and final position of reverse primer:

25 187509686, 187509848
 187509780, 187509957
 187509922, 187510077
 187509985, 187510157
 187510068, 187510242
 30 187510198, 187510363
 187510240, 187510402
 187510358, 187510528
 187516739, 187516905

	187516839, 187517007
	187516950, 187517116
	187517558, 187517725
	187517686, 187517856
5	187517722, 187517895
	187517847, 187518005
	187517899, 187518066
	187518000, 187518159
	187518082, 187518255
10	187518147, 187518315
	187518290, 187518451
	187518790, 187518955
	187518844, 187519010
	187519017, 187519169
15	187519125, 187519285
	187519235, 187519405
	187521006, 187521177
	187521066, 187521226
	187521171, 187521342
20	187521242, 187521412
	187521376, 187521545
	187521496, 187521665
	187522275, 187522435
	187522395, 187522567
25	187522457, 187522627
	187523994, 187524140
	187524081, 187524248
	187524279, 187524427
	187524386, 187524526
30	187524421, 187524566
	187524522, 187524666
	187524564, 187524737
	187524660, 187524834

	187524748, 187524922
	187524831, 187524973
	187524920, 187525063
	187524986, 187525139
5	187525062, 187525178
	187525498, 187525664
	187525619, 187525786
	187527123, 187527289
	187527218, 187527393
10	187527324, 187527496
	187530280, 187530448
	187530335, 187530508
	187530870, 187531020
	187530976, 187531145
15	187531040, 187531203
	187532465, 187532639
	187532511, 187532682
	187532635, 187532805
	187532720, 187532886
20	187532798, 187532940
	187534199, 187534339
	187534304, 187534429
	187534332, 187534502
	187534438, 187534606
25	187535251, 187535403
	187535344, 187535503
	187535400, 187535567
	187538056, 187538192
	187538141, 187538305
30	187538188, 187538352
	187538783, 187538918
	187538869, 187539025
	187538912, 187539080

	187539017, 187539189
	187539078, 187539239
	187539190, 187539350
	187539232, 187539400
5	187539355, 187539504
	187539394, 187539545
	187539501, 187539622
	187539540, 187539701
	187539627, 187539764
10	187539699, 187539849
	187539759, 187539920
	187539840, 187540016
	187539911, 187540065
	187540015, 187540181
15	187540073, 187540223
	187540184, 187540347
	187540236, 187540403
	187540345, 187540484
	187540400, 187540524
20	187540481, 187540606
	187540538, 187540679
	187540598, 187540772
	187540687, 187540831
	187540770, 187540913
25	187540830, 187540956
	187540925, 187541089
	187541016, 187541184
	187541085, 187541255
	187541183, 187541352
30	187541254, 187541424
	187541348, 187541504
	187541418, 187541580
	187541507, 187541669

	187541577, 187541726
	187541666, 187541809
	187541724, 187541893
	187541813, 187541942
5	187541887, 187542026
	187541943, 187542110
	187542025, 187542176
	187542120, 187542276
	187542186, 187542360
10	187542279, 187542400
	187542353, 187542509
	187542400, 187542562
	187542504, 187542668
	187542558, 187542717
15	187542661, 187542813
	187542760, 187542906
	187542806, 187542967
	187549198, 187549361
	187549316, 187549490
20	187549385, 187549556
	187549591, 187549765
	187549720, 187549886
	187549777, 187549937
	187549890, 187550054
25	187554804, 187554976
	187554897, 187555061
	187557084, 187557247
	187557176, 187557344
	187557249, 187557410
30	187557365, 187557537
	187557616, 187557791
	187557747, 187557889
	187557818, 187557984

	187557903, 187558071
	187557985, 187558149
	187560789, 187560948
	187560877, 187561020
5	187584406, 187584550
	187584482, 187584647
	187584544, 187584715
	187584650, 187584817
	187627676, 187627834
10	187627720, 187627886
	187627828, 187627971
	187627889, 187628050
	187627968, 187628133
	187628051, 187628214
15	187628126, 187628278
	187628217, 187628366
	187628288, 187628462
	187628360, 187628529
	187628459, 187628623
20	187628528, 187628691
	187628623, 187628792
	187628696, 187628862
	187628784, 187628927
	187628863, 187628989
25	187628920, 187629077
	187629015, 187629179
	187629069, 187629237
	187629181, 187629298
	187629229, 187629396
30	187629305, 187629458
	187629390, 187629561
	187629454, 187629600
	187629553, 187629694

	187629606, 187629728
	187629686, 187629836
	187629785, 187629925
	187629830, 187630003
5	187629924, 187630093
	187629997, 187630171
	187630090, 187630246
	187630182, 187630351
	187630276, 187630408
10	187630355, 187630518
	187630437, 187630574
	187630516, 187630682
	187630616, 187630785
	187630687, 187630853
15	187630782, 187630954
	187630858, 187631029

for the amplification of CDKN2A gene, each primer pair has respectively the following initial position of forward primer and final position of reverse primer:

20	21968149, 21968274
	21968651, 21968801
	21970835, 21971009
	21970962, 21971143
	21971106, 21971273
25	21974425, 21974592
	21974542, 21974709
	21974650, 21974814
	21994109, 21994285
	21994241, 21994398

30 for the amplification of TP53 gene, each primer pair has respectively the following initial position of forward primer and final position of reverse primer:

7572830, 7573002

7572907, 7573051
7573856, 7574026
7573989, 7574156
7576473, 7576588
5 7576544, 7576709
7576807, 7576971
7576927, 7577092
7577006, 7577176
7577097, 7577256
10 7577375, 7577531
7577486, 7577639
7578120, 7578282
7578239, 7578398
7578281, 7578453
15 7578403, 7578580
7579230, 7579398
7579327, 7579505
7579486, 7579616
7579596, 7579770
20 7579737, 7579874
7579817, 7579988

wherein said positions refer to the hg19 human reference genome.

14) Kit according to anyone of claims 11-13, said kit further comprises primers for detecting the following mutations in PIK3CA gene,
25 said mutations having the following COSMIC_id numbers:

12458
12459
12461
12463
30 12464
12580
12582
12584

	12590
	12591
	12592
	12597
5	13570
	13594
	17442
	17444
	17445
10	17449
	21451
	21462
	22540
	24712
15	24714
	249872
	249908
	25041
	25085
20	25086
	27133
	27134
	27155
	27156
25	27158
	27273
	27374
	27502
	27505
30	28938
	29110
	29313
	36285

	36286
	36289
	6147
	746
5	754
	757
	759
	760
	762
10	763
	764
	765
	766
	767
15	769
	770
	771
	772
	773
20	774
	775
	776
	777
	778
25	94984
	125368
	12579
	12589
	14052
30	163484
	166154
	21448
	230020

- 24710
24711
27490
27497
5 27499
39166
41785
51258
51259
10 6145
6149
6150
749
750
15 758
86045
86949
86950
87210
20 125370
94985
94986
94987
COSM87306
25 15) Kit according to anyone of claims 11-14, said kit comprising or consisting of a chip that comprises the primers defined in claims 13 and 14.