10.1148/radiol.2017162610

Appendix E1

FGln PET/CT Image Acquisition

PET/CT scans were obtained with a single Discovery 690 PET/CT scanner (GE Healthcare) with patients in the supine position. Scout radiographs were obtained followed by spiral CT scans spanning skull to proximal thigh regions with 140 kVp, 70 mA, a pitch of 1.75:1, reconstructed section thickness of 3.75 mm, and 0.8 second per rotation. CT data were reconstructed in a 512 × 512 matrix by using a filtered back-projection algorithm. FGln was administered in a single peripheral intravenous injection of 244 MBq ± 118 (<100 μg) quantum satis 5–10 mL in sterile water, administered in less than 30 seconds. Syringes and catheters were flushed with sterile water, removed, and radioassayed with a well counter for decay-corrected residual activity to determine net administered activity. PET of tumor region of interest was performed as dynamic imaging of a single bed position of 30 minutes duration beginning immediately before tracer injection, followed immediately by PET imaging spanning from skull to proximal thigh regions across multiple bed positions (five to seven bed positions total, depending on patient height) within a 15-minute period (approximately 2–3 minutes per bed). PET/CT was then performed 90 minutes ± 30 and 150 minutes ± 30 after injection by using the same CT acquisition parameters and with 45-minute PET spanning skull to proximal thigh regions across five to seven bed positions (6.4–9.0 minutes per bed). For patients receiving less than 185 MBq tracer (n = 9), 15–30 minutes of PET imaging was allocated to the single bed position of tumor region of interest, with the remaining 15–30 minutes of PET imaging time distributed equally to the other (2,21,33) bed positions. PET emission data were acquired in three-dimensional mode. PET images were reconstructed by using ordered subset expectation maximization iterative algorithm. Body PET images were reconstructed to 128 × 128 matrix over a 50-cm field of view (FOV). PET emission data for brain tumor region of interest was reconstructed to a 256 × 256 matrix over a 30 cm FOV. Emission data were corrected for random detector inhomogeneity, scatter, attenuation, dead time, and decay.

PET-based Analyses of FGln Radiotracer Biodistribution, Radiotracer Excretion, and Patient Dosimetry

To track the total administered activity after injection, the decay-corrected fraction of activity of the PET FOV spanning from skull to proximal thigh regions, denoted $a_{FOV,}$ was measured at each time point by using a digital three-dimensional volume of interest (VOI) that encompassed the skull-to-thighs PET FOV, denoted VOI_{FOV}. The average SUV (SUV_{ave}) within the VOI_{FOV} was multiplied by the volume (in cubic centimeters) of the VOI_{FOV}; the product was divided by patient body weight (in grams) to calculate the fraction of the administered tracer-dose present in the VOI_{FOV}, a_{FOV}. Because no activity exited the PET FOV by defecation or urination during the initial PET scanning time period, the decay-corrected a_{FOV} at the first time point was used as a normalization factor for the decay-corrected a_{FOV} values at all subsequent time points. As the lower extremities were partially excluded from the FOV, the a_{FOV} of the first PET scan (ie, the body PET scan immediately following the single-bed dynamic PET scan that began at the time of tracer injection) was used as a normalization factor, $a_{FOV,1}$, for the decay-corrected a_{FOV} in

PET scans at subsequent time points; for example, if no activity was eliminated from the body before the second scan, then $a_{FOV,2} \div a_{FOV,1} = 1.0$.

For organ activity-time measurements, tissue activity present in a selected digital organ VOI was quantified by using the SUV. SUV was calculated with the standard formula, as follows: [PET-measured activity (in becquerels) per milliliter of tissue of interest]/[total activity injected (in becquerels) ÷ total grams of body mass]. Activities within organs of interest were measured from the reconstructed PET images. Organs of interest included the brain, left cardiac ventricle wall, lungs, liver, spleen, pancreas, kidneys, small intestines, spine, humerus, and skeletal musculature. The compact bone of the peripheral skeleton demonstrated a relative paucity of activity, with skeletal activity predominantly visualized in the typical distribution of red marrow; thus, the humerus was considered representative of uptake in osteogenic cells and spinal uptake representative of red marrow uptake. Skeletal radiotracer uptake proved to be of key interest, as shall be discussed, potentially representing osseous uptake of free ^{18}F, as a radiometabolite; as a comparison, we measured skeletal uptake of free ^{18}F administered to a control group of cancer patients undergoing standard ^{18}F NaF bone PET scans, on the same PET/CT scanner. Activity measurements and dosimetric analyses involving the urinary bladder and gastrointestinal tract were analyzed. Each organ of interest was measured by using a contoured digital three-dimensional VOI within the organ anatomic boundaries to obtain the average SUV. Organ VOIs spanned at least three contiguous axial PET sections and encompassed at least 14 cm^3. Organ VOI contouring on PET data were guided by anatomic information provided by companion CT data, including PET/CT fusion display. Care was taken to ensure that the VOI of one organ of interest did not include activity from adjacent tissues. Anatomic sites demonstrating disease involvement at companion CT or available correlative imaging were excluded from normal organ VOI measurements. Organ activity was tabulated and graphed as ordinate data versus time after injection (in hours), on a software spreadsheet (Microsoft Office Excel 2007; Microsoft, Seattle, Wash). The graphed data were visually inspected to determine mathematical formula(e) to describe organ activity as a function of time, by using amplitude coefficient(s) and rate constant(s) (λ) terms that defined an activity-versus-time curve (ATC). Formula-derived organ activity values were tabulated as ordinate data versus time, alongside observed values. The numerical values of the pair(s) of coefficient and constant terms, for each formula, were determined by using a software program (Office Excel Solver plug-in, Microsoft) to minimize, overall, the differences between observed activity and derived-activity values; that is, to minimize the sum of the squares of the differences across all data time points. This "fitted" the ATC data to the observed data. ATC data were graphed for visual inspection of goodness-of-fit. Time-dependent changes in organ activity (corrected for physical isotope decay) reflected biologic processes, as quantified by the rate constant(s) (λ) terms of the ATC formulae. The clearance half-time ($t_{1/2}$) of λ term(s) describing a "falling" ATC were calculated as the rate constant (λ) divided by 0.693.

Quantification of tracer excretion was derived from PET image analysis. No hepatobiliary excretion was evident in any patient. Urinary excretion was evident in all patients. For purposes of urinary bladder dosimetry, the time required for half of all activity eliminated via the urinary bladder to be excreted into the urine, the urinary clearance half-time, CL, was estimated as follows: For each patient, a digital VOI was created that fully encompassed the urinary bladder on the three serial PET image datasets, $VOI_{b,t}$. The average SUV (SUV_{ave}) within the $VOI_{b,t}$ was multiplied by the volume (in cubic centimeters) of the $VOI_{b,t}$; the product was

divided by patient body weight (in grams) to calculate the fraction of the administered tracer dose present in the $VOI_{b,t}$, $a_{b,t}$. During the first PET scan (0–45 minutes, after injection), no activity exited the PET FOV and no urinary voiding occurred. Hence, any measured total activity in the $VOI_{b,t}$ during that period was the cumulative amount of activity entering into the urinary bladder up to that time point, with the following assumptions: no urinary tract reabsorption of excreted tracer, and the amount of tracer retention by urinary bladder tissues was negligible relative to the amount of excreted activity. At two subsequent PET time points, any activity absent from the body FOV was assumed to have been excreted isotope voided by the patient from the urinary bladder during the break in PET imaging (ie, assumed no tracer elimination by defecation, during the approximately 3-hour imaging time period). The decimal fraction of administered activity absent from body, due to voiding, was calculated as the difference between the total body activity currently ($a_{FOV,t}$) versus immediately after injection ($a_{FOV,1}$), as follows: $1–(a_{FOV,t} \div a_{FOV,1})$. The cumulative urinary excreta, at each time point, $a_{b,t}$, was calculated as the sum of both activity currently visualized within the bladder ($a_{FOV,t}$) plus activity already voided by the urinary bladder, calculated as above. Cumulative urinary bladder activity was tabulated and graphed as ordinate data versus time after injection; this fractional value, at the approximately 3-hour time point, represented the fraction of administered isotope excreted via the urinary bladder, f_u (assuming subsequent urinary excretion was negligible). As described for organs of interest ATC, a mathematical formula was selected to describe cumulative urinary bladder activity as a monoexponential function of time. The rate constant of that exponential expression, λ_u, served to describe the clearance of activity by the kidneys into the urine, CL, serving as input data for OLINDA, where $CL = 0.693 \div \lambda_u$.

For radiation dosimetry calculations, for each organ of interest, other than the urinary bladder and gastrointestinal tract, the following approach was used to determine each organ-specific, time-integrated activity coefficient, $\tilde{a}(r_S, T_D)$. The extrapolated ATC function of each organ was integrated, from $t = 0$ to $t = \infty$, to find its area under the curve (AUC); the AUC indicating the $\tilde{a}(r_S, T_D)$ for that organ. The $\tilde{a}(r_S, T_D)$ is expressed in units of seconds (48) but can be also understood as percentage injected dose · second or megabecquerel · hour/MBq; the latter is the form used by OLINDA/EXM (OLINDA/EXM v.1, Vanderbilt University, Nashville, Tenn), a U.S. Food and Drug Administration–approved radiation dosimetry software package (49). The $\tilde{a}(r_S, T_D)$ value, understood in units of megabecquerel · hour/ megabecquerel, indicates the number of radioactive disintegrations (1 MBq · hour = 3.6×10^9 decays) that occurred in an organ of interest, per megabecquerel of administered activity. Numerical integration of an ATC function was compared with the result obtained with the trapezoidal method of finding the AUC, for a (r_S, t) organ data. For trapezoidal method, the AUC beyond the time point of the last experimental datum was calculated one of two ways: *(a)* if the ATC had reached a plateau, activity was assumed to disappear only by physical decay; or *(b)* if the ATC indicated ongoing biologic clearance, at the final experimental time point, then the AUC region beyond was calculated by numerical integration, extrapolating from the last time point to $t = \infty$, by using the coefficient and biologic λ constant of the exponential term that predominantly defined the terminal portion of the fitted ATC. In none of these organs of interest did the ATC rise at the final experimental time point. The experimentally derived numbers of disintegrations occurring in each organ per unit administered activity, N_{organ}, were calculated as described and used as input data for OLINDA, in calculation of absorbed dose, D, and effective dose, E. OLINDA allows users to select from libraries of data describing anthropomorphic phantom models and radioactive isotope decay characteristics to generate a dose factor, DF, toward calculation of the

standard phantom model, named "Adult Male." Note that the standard "Adult Male" phantom model contains organs specific to the female (eg, ovaries and uterus) as well as to the male (eg, prostate gland and testes) (50). The standard Voiding Bladder Model, as implemented in OLINDA, was used to predict human urinary bladder dosimetry (51) from experimental f_u and CL values, obtained as described, with a bladder-voiding interval chosen as 4.5 hours. Hepatobiliary fecal excretion was negligible. The total number of disintegrations associated with a single administered megabecquerel of ^{18}F is the inverse value of its physical constant, $1 \div \lambda_P$; for ^{18}F, this is 2.6455 hours or MBq · hour/MBq, which shall be referred to as N_{total}. The $\tilde{a}$ (r_S, T_D) values (in megabecquerel · hour/ megabecquerel) of organs of interest were summed, in each patient, as N_{organs}. OLINDA, using its implementations of the Voiding Bladder Model and ICRP 30 GI Model, calculated the disintegrations and dosimetric contributions associated with radioactive excreta in the urinary bladder and portions of the gastrointestinal tract, N_{excreta}. Subtracting N_{organs} and N_{excreta} from N_{total} indicated the remaining amount of disintegrations from the administered dose that required accounting, $N_{\text{remainder}}$, in megabecquerels · hour/ megabecquerels, which were assumed to be uniformly distributed among remaining bodily tissues (ie, neither organs of interest nor excretory route organs.) Experimental data from each patient was analyzed separately to achieve measurements of absorbed dose, D, and effective dose, E.

Plasma Radioassay for Blood Clearance and Radiometabolite Analyses

Activity in whole blood and plasma specimens was radioassayed with a well counter (1480 WIZARD 3 Automatic Gamma Counter; PerkinElmer, Shelton, Conn), after separating blood and plasma by centrifuge (4000 rpm for 10 minutes at 4°C). Well counting obtained a minimum of 10,000 counts per specimen correcting for background activity and radioactive decay from time of tracer injection. An ^{18}F standard was assayed on the well counter to obtain a calibration factor for converting sample scintillation counts to becquerels of activity. Dividing sample activity by the sample volume and intravenously administered patient dose (in becquerels) quantified the sample activity in terms of fraction of injected dose (expressed as a decimal) per milliliter (injected dose per milliliter). The injected dose per milliliter value was multiplied by the patient body weight (in grams) to convert to SUV for comparison with tumor SUV values measured with PET, also calculated as the injected dose per milliliter tissue volume, normalized to patient body weight (in grams). Blood and plasma tracer concentration data were separately plotted versus time. A mathematical function curve was fit to the experimental data with a least-squares approach, with goodness-of-fit assessed with conventional graphic and coefficient analyses. From the patient-specific fitted curve formulae, individual blood and plasma clearance times were determined. Plasma protein binding of tracer was determined by ultracentrifugation of plasma specimens (as below) followed by well-counting of activity in supernatant versus pellet.

High-performance liquid chromatography (HPLC)–based radiometabolite analysis was variably limited by low-count statistics in plasma specimens. Iterative methodologic modifications were explored in this trial toward optimizing radio HPLC methodology, limiting comparison of radio HPLC data among patients. For the data presented, for radio HPLC of blood aliquots, serum proteins were precipitated with acetontrile (vol/vol = 1/1), then the samples were centrifuged for 5 minutes at 15 000 rpm. The supernatant was removed by filtration with a 0.45-

um filter. Samples were then completely evaporated by using a Biotage V-10 Evaporator (Biotage, Charlotte, NC) and resuspended in 300 μL of mobile phase for HPLC injection. The resuspended supernatant was characterized with an analytical chiral HPLC (Chirex 3126 (d)-penicillamine; mobile phase: 1 mmol/L $CuSO_4$ solution; flow rate: 1 mL/min; room temperature). Radio HPLC of an ^{18}F reference standard was performed under the same conditions to define a characteristic elution time for an anticipated ^{18}F radioactive peak. Elution fractions were collected every 60 seconds and measured on the gamma counter (WIZARD 3 1480 γ-counter; PerkinElmer, Waltham, Mass). The activity of each radio HPLC peak was quantified as a percentage of the total activity in the HPLC sample (cpm-peak ÷ cpm-sample × 100, where cpm is counts per minute), to express the amount of intact FGln or radiolabeled metabolite each as a relative percentage of total. The relative amount of each radioactive peak (parent compound or radiometabolite) at each sampling time point was calculated by dividing the activity-time integral of an individual radiopeak by the activity-time integral of the total tracer. Parent tracer and radiometabolites were converted to injected dose per milliliter plasma, as above.

^{18}F NaF Bone PET

To obtain context for the relative amount of skeletal radiotracer uptake—representing uptake of free ^{18}F radiometabolite, at least in part (see Discussion)—visualized with FGln PET in the study population, the skeletal uptake of exogenous free ^{18}F, administered as ^{18}F NaF, was quantified from ^{18}F NaF PET bone scans obtained as standard of care in a control group of patients with prostate cancer (n = 10) without evidence of humeral or spinal metastatic disease. Retrospective analysis of ^{18}F NaF PET bone scans obtained as part of a separate institutional review board–approved study protocol with waiver of informed consent. NaF PET scans were performed by using the same PET/CT scanner used for the FGln PET scans, a single Discovery 690 PET/CT scanner (GE Healthcare). PET/CT scans were obtained approximately 1 hour (mean, 71 minutes ± 12) after injection of 222 MBq ^{18}F NaF intravenously. Before tracer administration, a scout radiograph was obtained, followed by CT data spanning axial planes from skull to proximal thigh regions, acquired with 120 kVp, 70 mA, a pitch of 1.75:1, reconstructed section thickness of 3.75 mm, and 0.8 second per rotation. CT data were reconstructed in a 512 × 512 matrix by using a filtered back-projection algorithm. PET imaging spanned from skull vertex to feet across multiple bed positions (including again the bed position used for dynamic PET imaging), 2–3 minutes per bed. PET emission was performed in three-dimensional mode. PET images were reconstructed by using an ordered subset expectation maximization iterative algorithm. PET images were reconstructed to a 128 × 128 matrix over a 50-cm FOV. Emission data were corrected for random detector inhomogeneity, scatter, attenuation, dead time, and decay. PET, CT, and fusion PET/CT images shown were generated for display and analysis by using an integrated GE PACS AW Suite Workstation (GE Healthcare). Average SUVs were measured in the spine and humerus by means of digital VOI.

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Table E1: FGln Plasma Radioassay Identify FGln Metabolism and Incorporation of Radiotracer into Circulating Polypeptides in Plasma

	Radiotracer Incorporation into Plasma Pellet	
	Percentage of Total Tracer Activity in Plasma	Absolute Amount of Activity in Plasma Specimen (% Injected Dose per Milliliter)

Parameter	1 Minute	5 Minutes	15 Minutes	30 Minutes	1 Minute	5 Minutes	15 Minutes	30 Minutes
Supernatant	90 ± 4.2	86 ± 3.6	84 ± 3.6	73.9 ± 7.0	0.0086 ± 0.0046	0.0056 ± 0.0010	0.0040 ± 0.0008	0.0031 ± 0.0009
Pellet	10 ± 4.2	14 ± 3.6	16 ± 3.6	26.1 ± 7.0	0.0008 ± 0.0006	0.0009 ± 0.0004	0.0008 ± 0.0002	0.0008 ± 0.0002

Plasma Radiometabolites (% of Total Plasma Activity)

	Supernatant				Supernatant + Pellet	
Parameter	1 Minute	5 Minutes	15 Minutes	30 Minutes		30 Minutes
Parent FGln	85.6 ± 19.3	88.1 ± 9.3	80.6 ± 7.6	65.0 ± 21.0	Total Radiometabolites	52.6 ± 22.5
^{18}F (free) metabolite	12.6 ± 19.9	10.6 ± 9.7	18.3 ± 8.8	32.6 ± 20.2		
Other radiometabolites	1.7 ± 17.6	1.3 ± 4.0	1.1 ± 3.3	2.3 ± 5.9		

Note.—Data shown are relative amounts of FGln parent compound and radiometabolites in plasma supernatant at 1, 5, 15, and 30 minutes after tracer injection (population averages ± standard deviations). Total activity in blood and plasma specimens was radioassayed at multiple time points. Parent and metabolites were separated by radio HPLC, identified by characteristic radio HPLC elution times. The amount of each radio HPLC–eluted peak was quantified by scintillation well counting, correcting for background activity and physical decay. Relative amounts were calculated as percentage of sum of total activity of identified radio HPLC peaks in each sample. Plasma protein was separated into pellet by ethanol precipitation and ultracentrifuge; plasma supernatant versus pellet activities was counted. Study was performed in a subset of patients (n = 5). The absolute amount of tracer in pellet (% injected dose per milliliter of plasma) appeared unchanged at 30 minutes versus 1 minute (P = not significant) as tracer cleared from plasma supernatant. Total plasma radiometabolites (at 30 minutes) represents the sum of radiometabolites in plasma supernatant and incorporated into plasma polypeptides (pellet activity).

Table E2: FGln Biodistribution and Patient Demographics in Fasting and Nonfasting Patients

Parameter	Fasting Patients (n = 13)	Nonfasting Patients (n = 12)	P Value
Blood pool	1.5 ± 0.4	1.4 ± 0.2	.93
Bone marrow	3.2 ± 1.1	4.2 ± 0.8	.006
Osteogenic cells	1.1 ± 0.3	1.1 ± 0.3	.98
Brain	0.4 ± 0.1	0.5 ± 0.2	.33
Heart wall	1.9 ± 0.6	2.2 ± 0.2	.10
Liver	2.9 ± 0.8	2.7 ± 0.7	.63
Lungs	0.7 ± 0.4	0.5 ± 0.2	.10
Skeletal muscle	1.1 ± 0.2	1.2 ± 0.2	.13
Spleen	1.7 ± 0.4	1.9 ± 0.3	.40
Pancreas	3.1 ± 1.2	2.8 ± 0.7	.72
Small intestines	2.1 ± 0.6	2.0 ± 0.4	.70
Tumor SUV_{peak}	2 ± 1.2	2.6 ± 0.8	.09
Tumor:blood pool	1.4 ± 1.1	1.9 ± 0.7	.08
Age (y)	51.5 ± 12.7	51.4 ± 19	.99
Sex*			.16
F	9	5	
M	4	7	

Note.–Expect where indicated, data are mean radiotracer uptake values ± standard deviations in organs ($SUV_{average}$) and tumors (SUV_{peak} and tumor-blood pool ratio) at 1 hour after tracer injection, in fasting and nonfasting patients. Patient demographics include age and sex. Statistical tests (see Methods) identified no differences between groups, except a possible slight difference in bone marrow uptake; if a standard Bonferroni correction for multiple comparisons is applied, then the P value for the bone marrow comparison (P = .006) is not considered significant.

* Data are numbers of patients.

Table E3: Plasma Levels of Amino Acids and Organic Chemical Compounds in Fasting and Nonfasting Patients

Amino Acid	Fasting Patients (*n* = 13)	Nonfasting Patients (*n* = 12)	*P* Value
Aspartic acid	4.3 ± 1.3	3.7 ± 1.6	.229
Glutamic acid	49.8 ± 17.5	74.9 ± 32.6	.010*
Hydroxyproline	15.1 ± 6.7	15.0 ± 7.3	.490
Serine	100.0 ± 19.1	115.0 ± 37.6	.887
Asparagine	39.6 ± 16.6	50.6 ± 20.8	.169
Alpha-amino adipic acid	1.5 ± 1.2	0.9 ± 1.5	.390
Glycine	245.2 ± 66.9	282.9 ± 86.3	.529
Glutamine	599.2 ± 144.3	632.5 ± 162.4	.639
Sarcosine	1.3 ± 0.8	1.0 ± 1.1	.821
Beta-alanine	2.5 ± 1.8	6.7 ± 8.0	.706
Taurine	61.1 ± 11.4	56.3 ± 15.2	.076
Histidine	70.0 ± 10.5	84.5 ± 35.6	.849
Citrulline	29.1 ± 7.8	28.4 ± 10.0	.237
Arginine	74.6 ± 14.9	74.2 ± 29.8	.595
Threonine	118.3 ± 26.4	144.3 ± 38.7	.141
Alanine	350.8 ± 97.6	418.8 ± 123.5	.292
1-Methylhistidine	8.0 ± 10.0	17.3 ± 17.0	.267
Gamma-amnio butyric acid	0.3 ± 0.4	0.2 ± 0.2	.999
3-Methylhistidine	2.8 ± 1.7	5.1 ± 1.9	.015*
Beta-amnio isobutyric acid	1.8 ± 1.1	2.3 ± 1.0	.165
Proline	168.6 ± 42.4	247.9 ± 79.9	.026*
Ethanolamine	7.3 ± 1.6	5.1 ± 1.8	.003*
Alpha-amino butyric acid	26.3 ± 8.6	24.2 ± 8.4	.117
Tyrosine	58.7 ± 9.5	79.8 ± 26.7	.028*
Valine	227.7 ± 53.2	253.7 ± 93.1	.684
Methionine	22.2 ± 4.0	26.1 ± 5.5	.136
Cystathionine	0.2 ± 0.3	0.3 ± 0.7	.999
Isoleucine	68.2 ± 21.3	86.9 ± 29.8	.057
Leucine	126.1 ± 38.1	143.3 ± 49.3	.481
Homocystine	0.1 ± 0.0	0.1 ± 0.0	.999
Phenylalanine	60.3 ± 5.9	69.9 ± 17.5	.021
Tryptophan	50.9 ± 9.9	61.7 ± 19.2	.283
Ornithine	72.8 ± 16.9	86.0 ± 21.5	.512
Lysine	173.1 ± 30.4	199.1 ± 50.8	.834

Note.—Data are population values (arithmetic means ± standard deviation) between groups and are in micromoles per liter.

* Statistically significant with standard *t* test. If a standard Bonferroni correction for multiple comparisons is applied, then no values differ significantly between groups.

Table E4: Detailed Description of Patient and/or Tumor-specific Gene Alterations Identified in Tumor Specimens of Study Participants (see Table 1)

Patient No.	Tumor DNA Alterations
1	**SDHB** E176× germline mutation TERT (NM_198253) promoter variant (g.1295228C > T)
2	**SDHB** (c 19_41dup23 frameshift mutation) germline mutation BCOR (NM_001123385) exon4 p.K530M (c.1589A > T)

3	**FH** F402LFsX3 (c 1209delT), germline mutation
4	**TP53** (NM_000546) exon6 p.Y220× (c.660T > G) KDM5A (NM_001042603–12p13.33) amplification (fold change: 2.8)
	ETV6 (NM_001987–12p13.2) amplification (fold change: 2.9)
	CDKN1B (NM_004064–12p13.1) amplification (fold change: 2.9)
	CDKN2Ap14ARF (NM_058195) exon2 p.A77_P80delinsL (c.229_239delinsCT)
	CDKN2Ap16INK4A (NM_000077) exon1 p.G35 V (c.104G > T)
	CDKN2Ap16INK4A (NM_000077) exon2 p.L62_H66delinsY (c.186_196delinsCT)
	NF1 (NM_001042492) exon23 p.D1028Y (c.3082G > T)
	NOTCH1 (NM_024408) Deletion: c.155+12081_c.4860–25del
5	ARID1A (NM_006015) exon1 p.S90fs (c.267_295delCAGCGGCGGCGGGCCCGGCGCGGAGCCGG)
	ATM (NM_000051) exon36 p.Q1825 (c.5473C > T)
	CDH1 (NM_004360) exon10 p.E482* (c.1444G > T)
	DOT1 L (NM_032482) exon12 p.S327C (c.980C > G)
	ERG (NM_182918) exon8 p.Q289* (c.865C > T)
	FOXA1 (NM_004496) exon2 p.L193 V (c.577C > G)
	RHOA (NM_001664) exon3 p.G62R (c.184G > A)
6	ESR1 (NM_001122740) exon9 p.D538G (c.1613A > G)
7	ESR1 (NM_001122740) exon9 p.D538G (c.1613A > G) **GATA3** (NM_002051) exon6 p.S407fs (c.1219_1220delTC)
	MLL3 (NM_170606) exon52 p.R4549C (c.13645C > T)
	RAD51B (NM_133509) exon11 p.Q371* (c.1111C > T)
8	NA
9	**PIK3CA** (NM_006218) exon2 p.R38C (c.112C > T) **TP53** (NM_000546) exon4 p.E68fs (c.203_204delAG)
	APC (NM_000038) exon16 p.R1435fs (c.4303_4321delAGAAGTAAAACACCTCCAC)
	FBXW7 (NM_033632) exon9 p.R465C (c.1393C > T)
10	NA Sequenom mass spectrometry genotyping for specific mutations in eight genes (see Methods): **KRAS** G12 V (c.35 G > T)
11	NA
12	NA

13	NA
14	FoundationOne **KRAS** G12D **CDKN2A/B** deletion **TP53**V218fs*4 MAK2 K4 deletion
15	NA
16	**SDHA** amplification (fold change: 2.1) TERT amplification (fold change: 2.1) **PIK3C2G** amplification (fold change: 3.3) **KRAS** amplification (fold change: 3.0) H3F3C amplification (fold change: 2.3) CCNE1 amplification (fold change: 6.6)
17	NA
18	NA
19	NA
20	NA
21	NA
22	NA
23	**IDH1** (NM_005896) exon4 p.R132H (c.395G > A) CIC (NM_015125) exon11 p.L942fs (c.2820_2823dupGCCA) ETV112 (NM_001163147) exon6 p.P95fs (c.283_284insT) TERT (NM_198253) promoter variant (g.1295228C > T)
24	**IDH1** (NM_005896) exon4 p.R132S (c.394C > A) **TP53** (NM_000546) exon6 p.H214R (c.641A > G) **SOX2** 13 (NM_003106–3q26.33) amplification (fold change: 2.8) **SDHA**F214 (NM_017841–11q12.2) amplification (fold change: 2.3) ATRX (NM_000489) exon22 p.Y1820fs (c.5460_5484delTACAGCATTAACAAAATTCTTGCCT) FAT1 (NM_005245) exon2 p.L562F (c.1686G > T) KDM6A (NM_021140) exon26 p.I1292S (c.3875T > G)
25	**TP53** (NM_000546) exon8 p.V272 L (c.814G > T)

SOX2 (NM_003106–3q26.33) amplification (fold change: 3.0)

ARID5B (NM_032199–10q21.2) deletion (fold change:–2.6)

PTEN (NM_000314–10q23.31) deletion (fold change:–1.7)

PTEN (NM_000314) exon5 p.R130Q (c.389G > A)

RB1 (NM_000321) exon15 splicing variant (c.1421+2T > A)

ROS1 (NM_002944) exon40 p.V2089M (c.6265G > A)

TERT (NM_198253) promoter variant (g.1295228C > T)

Note.—Several of the genes have been reported to influence tumor utilization of glutamine. Selected examples (indicated above by bold font) with citations are as follows: *SDH* (2), *FH* (2), *TP53* (21), *ETV6* (36), *CDK* (15), *NF1* (17), *NOTCH1* (16), *CDH1* (37), *FOXA1* (38), *RHOA* (39), *GATA3* (40), *PIK3CA* (41), *APC* (42), *KRAS* (43), *IDH* (44), *SOX2* (45), *PTEN* (46), *RB1* (47). Note that other genes listed above (not in bold font) may affect glutamine metabolism as well. Tumor genetic alternations were detected with MSK-IMPACT unless otherwise specified. NA = no tumor genetic assays were performed.