Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Ho AL, Grewal RK, Leboeuf R, et al. Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. N Engl J Med 2013;368:623-32. DOI: 10.1056/NEJMoa1209288

SUPPLEMENTARY APPENDIX

Table of Contents

Alan L. Ho, Ravinder K. Grewal, Rebecca Leboeuf, Eric J. Sherman, David G. Pfister, Desiree Deandreis, Keith S. Pentlow, Pat B. Zanzonico, Sofia Haque, Somali Gavane, Ronald A. Ghossein, Julio C. Ricarte-Filho, José M. Domínguez, Ronglai Shen, R. Michael Tuttle, Steven M. Larson and James A. Fagin

Supplementary Methods

Other Protocol Inclusion Criteria

- Patients ≥ 18 years-old
- Pathology specimen from the original tumor (thyroid) and/or metastases must be available for genotyping.
- Evaluable disease by RECIST v.1.1
- Female patients will need to be post-menopausal or have a negative serum pregnancy test if pre-menopausal.
- Female patients of child-bearing potential must agree to use reliable methods of contraception for the duration of the study and until 4 weeks after the last dose of selumetinib.
- Male patients with sexual partners who are pregnant or who could become pregnant (i.e., women of child-bearing potential) must agree to use acceptable methods of contraception for 16 weeks after completing the study to avoid pregnancy and/or potential adverse effects.
- ANC $\geq$ 1.5x$10^{9/}$L (1500 per mm^3), platelets $\geq$ 100 x 10^9/L (100,000 per mm^3), and hemoglobin $\geq$ 9 g/dl
- ALT/SGOT and AST/SGPT < 2.5 X upper limit of normal (ULN) unless the patient has liver metastases. If the patient has liver metastases, ALT/SGOT and AST/SGPT must be < 5 X upper limit of normal (ULN).
- Bilirubin ≤ 1.5 X ULN
- Serum creatinine clearance >50 ml/min, by either Cockcroft-Gault formula or 24-hour urine collection analysis.
- Ability to understand and the willingness to sign a written informed consent document.

Protocol Exclusion Criteria

- Patients who are receiving any other investigational agents.
- Patients may not have any prior exposure to MEK, Ras, or Raf inhibitors.
- Patients unable to follow a low iodine diet, patients requiring medication with high content in iodide (amiodarone), or patients receiving IV iodine containing contrast as part of radiographic procedure.
- Patients with clinically significant cardiovascular disease as defined by the following:
    - Baseline LVEF ≤ 50%
    - Heart failure NYHA Class II or above
    - Uncontrolled hypertension (BP ≥150/95 despite optimal therapy)
    - Prior or current cardiomyopathy
    - Arial fibrillation with heart rate >100 bpm
    - Unstable ischemic heart disease (MI within 6 months prior to starting treatment, or angina requiring use of nitrates more than once weekly)
- Patient with known hypersensitivity to Thyrogen (human recombinant thyrotropin).
- Patients with a history of allergic reactions attributed to compounds of similar chemical or biologic composition to agents used in the study.
- Uncontrolled undercurrent illness including, but not limited to, ongoing or active infection or psychiatric illness/social situations that would limit compliance with study requirements.
- Pregnant women will be ineligible; breast feeding should be discontinued if the mother is treated with selumetinib.
- Brain metastases or spinal cord compression unless treated and stable (for at least 3 months) off steroids.
- Mean QTc interval >450 ms

Note on the statistical design

This pilot study was originally designed to evaluate 10 *BRAF* mutant (MUT) and 10 *BRAF* wild-type (WT) patients, in order to determine if change in RAI uptake after selumetinib was related to tumor genotype. The stratification by genotype was eliminated during the conduct of the study to facilitate enrollment and avoid excluding patients who could benefit. The primary endpoint was therefore amended to only determine the percentage of patients with selumetinib-induced increases in iodine incorporation of index tumor(s) irrespective of tumor genotype. Nine *BRAF* MUT and 11 *BRAF* WT patients were ultimately evaluable upon study completion.

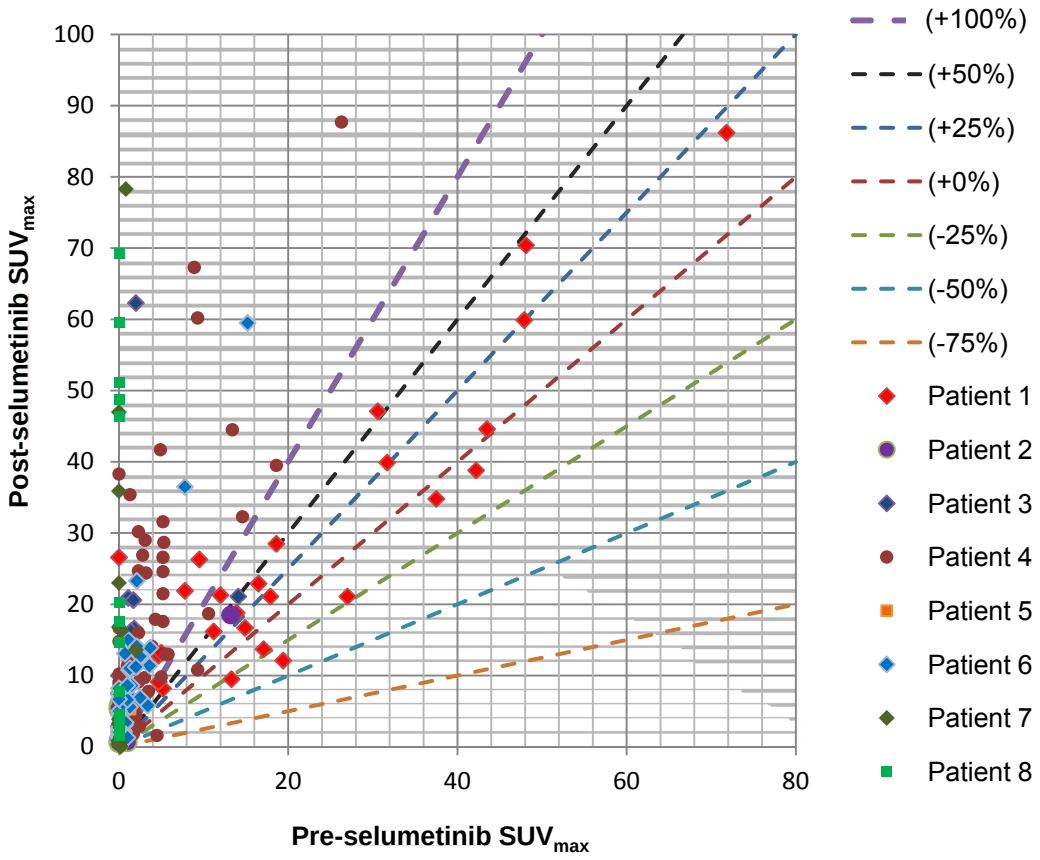


Figure S1. Quantitative lesional iodine incorporation before and after selumetinib detected by ^{124}I PET/CT for every metastasis in all 8 patients who received RAI.

The dashed lines mark points on the graph corresponding to different degrees of change in lesional SUV_{max} after selumetinib. The central dashed line demarcates no change in iodine incorporation following selumetinib (0%). Dashed lines to the left of the central line represent graded percentage increases in ^{124}I incorporation (+25%, +50%, +100%), while the lines to the right represent graded percentage decreases (-25%, -50%, -75%).

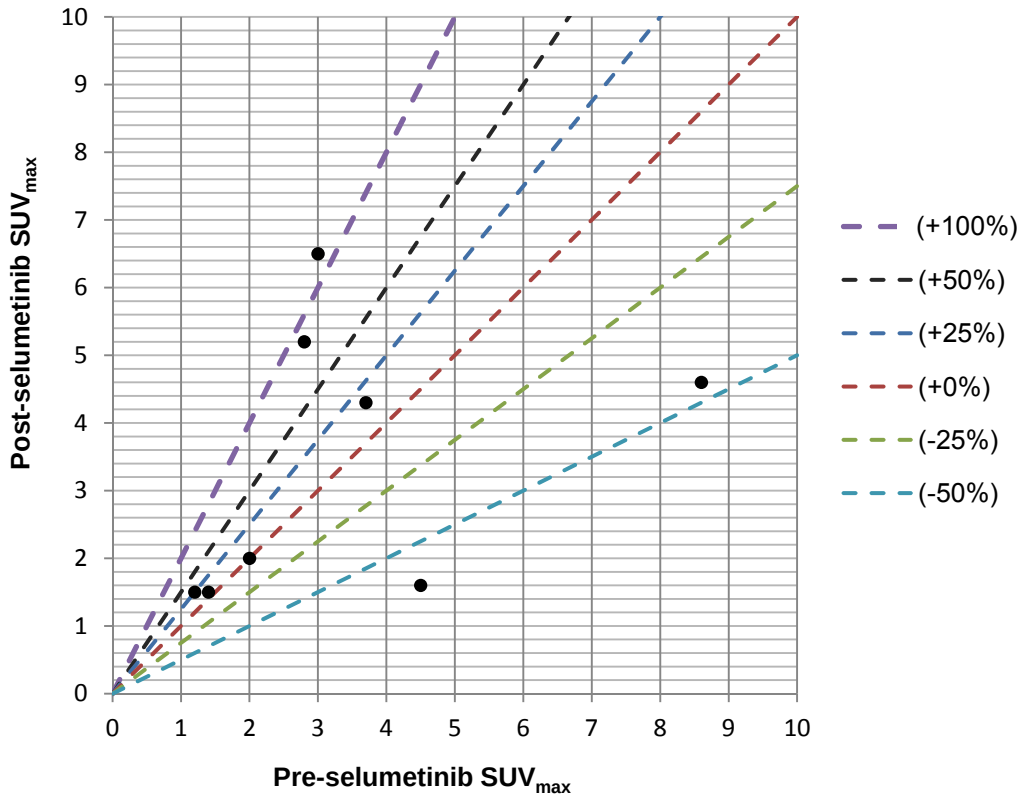
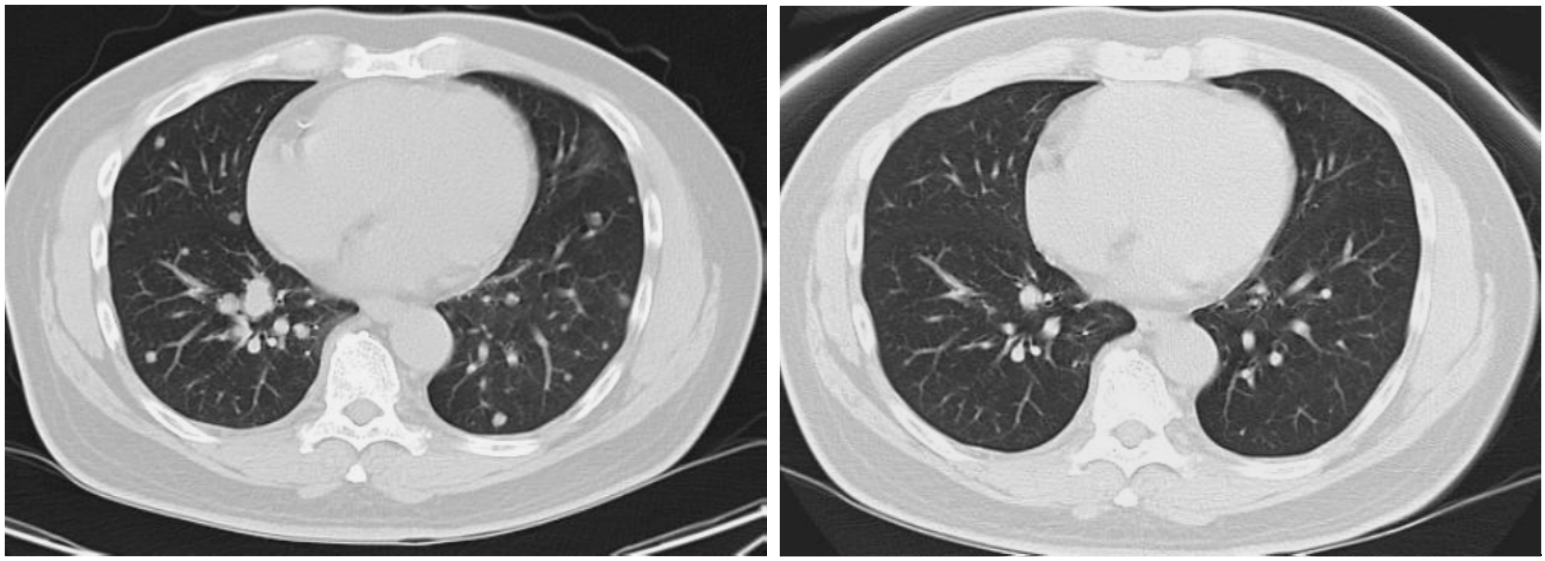


Figure S2. Quantitative iodine incorporation before and after selumetinib in normal salivary tissue. Each point on the graph represents the pre- and post-selumetinib ^{124}I PET SUV_{max} values measured in the normal parotid or submandibular gland from a single patient. The dashed lines mark points corresponding to different degrees of change in salivary SUV_{max} after selumetinib. The central dashed line demarcates no change in iodine incorporation following selumetinib (0%). Dashed lines to the left of the central line represent graded percentage increases (+25%, +50%, +100%), whereas lines to the right represent graded percentage decreases (-25%, -50%). Minimal changes in salivary SUV_{max} were noted.



Baseline **2 months after selumetinib and RAI**

Figure S3. Reduction in an *NRAS* MUT patient's bilateral lung metastases two months after treatment with selumetinib and RAI.

Table S1: Primer Sequences Used for Detection of Fusion Oncogenes.

Name	Sequence (5´-3´)
RET exon 10 Forward	CACCTGCAACTGCTTCCCTGAGGA
RET exon 11 Reverse	CGGCACAGCTCGTCGCACAGT
RET exon 12 Forward	GCAACGGCCTTCCATCTGAA
RET exon 13 Reverse	ACTCGGGGAGGCGTTCTCTTT
PAX8 exon 7/PPARG Forward	AACCTCTCGACTCACCAGAC
PAX8 exon 8/PPARG Forward	CCCTTCAATGCCTTTCCCC
PAX8 exon 9/PPARG Forward	CTATGCCTCCTCTGCCATC
PAX8 /PPARG All Reverse	AGAATGGCATCTCTGTGTCAAC
GAPDH Forward	CCATGGTGTCTGAGCGATGT
GAPDH Reverse	CAGCCGCATCTTCTTTTGC

Table S2: Selumetinib Effects on Iodine Incorporation and Tumor Response to RAI Therapy According to Tumor Histology

Tumor Histology	Patients with increased lesional iodine incorporation after selumetinib (fraction of total)	Patients who received RAI (fraction of total)	Best Overall Response by RECIST after RAI
Papillary (Classical)	3 (3/5)	2 (2/5)	1 cPR, 1 SD
Papillary (Tall Cell Variant)	3 (3/8)	0 (0/8)	N/A
Poorly Differentiated	6 (6/7)	6 (6/7)	4 cPR, 2 SD
Totals	**12 (12/20)**	**8 (8/20)**	**5 cPR, 3 SD**

cPR, confirmed partial response; *SD,* stable disease.

N/A, Not available

Table S3: Selumetinib Effects on Iodine Incorporation and Tumor Response to RAI Therapy According to Criteria Used to Define RAI-refractoriness

Criteria for RAI-refractoriness*	Patients with increased lesional iodine incorporation after selumetinib (fraction of total)	Patients who received RAI (fraction of total)	Best Overall Response by RECIST after RAI
1. Non-RAI avid lesion on a diagnostic RAI scan (within 2 years of study enrollment)	1 (1/4)	1 (1/4)	1 cPR
2. RAI-avid lesion that remained stable in size or progressed despite RAI 6 months or more prior to study entry	2 (2/2)	1 (1/2)	1 cPR
3. FDG-avid lesion on PET scan	9 (9/17)	6 (6/17)	3 cPR, 3 SD
4. Non-RAI avid lesion on an RAI scan (within 5 years of study enrollment)**	3 (3/7)	2 (2/7)	2 cPR

* Note that several patients qualified for the study by more than one criterion.

**This criterion was not a part of the study eligibility criteria (it includes all patients that qualified by Criterion #1).

cPR, confirmed partial response; *SD,* stable disease.

Note: 10 of the 20 patients on the study had no detectable ^{124}I incorporation on the baseline ^{124}I PET scan.

Table S4: All Adverse Events RELATED (possibly, probably, or definitely related) to Selumetinib

Adverse Event	Grade 1/2	Grade 3	Grade 4	Total*
Rash	18	0	0	18 (90%)
Fatigue	16	0	0	16 (80%)
Hepatic enzymes increased	14	0	0	14 (70%)
Diarrhea	9	0	0	9 (45%)
Nausea	8	0	0	8 (40%)
Mucositis oral	7	0	0	7 (35%)
Edema limbs	6	0	0	6 (30%)
Constipation	4	0	0	4 (20%)
Hypoalbuminemia	3	0	0	3 (15%)
Pain	3	0	0	3 (15%)
White blood cell decreased	3	0	0	3 (15%)
Dry mouth	2	0	0	2 (10%)
Dry skin	2	0	0	2 (10%)
Edema face	2	0	0	2 (10%)
Hyperglycemia	2	0	0	2 (10%)
Hypocalcemia	2	0	0	2 (10%)
Abdominal distension	1	0	0	1 (5%)
Bronchopulmonary hemorrhage	1	0	0	1 (5%)
Dysphagia	1	0	0	1 (5%)
Dyspnea	1	0	0	1 (5%)
Eye disorders - Other, specify	1	0	0	1 (5%)
Hoarseness	1	0	0	1 (5%)
Hypernatremia	1	0	0	1 (5%)
Hypertension	1	0	0	1 (5%)
Hyponatremia	1	0	0	1 (5%)
Periorbital edema	1	0	0	1 (5%)
Platelet count decreased	1	0	0	1 (5%)
Vomiting	1	0	0	1 (5%)
Total:	113	0	0	

*****The values in the parentheses represent the percentage of patients experiencing the indicated adverse event.

Note: There were no Grade 5 events reported.

Table S5: All Adverse Events UNRELATED (unrelated or unlikely related) to Selumetinib

Adverse Event	Grade 1/2	Grade 3	Grade 4	Total*
Hyperglycemia	14	0	0	14 (70%)
Hypoalbuminemia	12	0	0	12 (60%)
Hypertension	4	4	0	8 (40%)
Fatigue	7	0	0	7 (35%)
Hypocalcemia	7	0	0	7 (35%)
Cough	7	0	0	7 (35%)
Pain	7	0	0	7 (35%)
White blood cell decreased	6	0	0	6 (30%)
Creatinine increased	5	0	0	5 (25%)
Peripheral Neuropathy	5	0	0	5 (25%)
Constipation	4	0	0	4 (20%)
Platelet count decreased	4	0	0	4 (20%)
Alkaline phosphatase increased	3	0	0	3 (15%)
Dizziness	3	0	0	3 (15%)
Dry mouth	3	0	0	3 (15%)
Hepatic enzymes increased	3	0	0	3 (15%)
Hyperkalemia	3	0	0	3 (15%)
Hyponatremia	3	0	0	3 (15%)
Nausea	3	0	0	3 (15%)
Anemia	2	0	0	2 (10%)
Dysphagia	2	0	0	2 (10%)
Edema limbs	2	0	0	2 (10%)
Hypernatremia	2	0	0	2 (10%)
Hypoglycemia	2	0	0	2 (10%)
Palpitations	2	0	0	2 (10%)
Alopecia	1	0	0	1 (5%)
Blood bilirubin increased	1	0	0	1 (5%)
Diarrhea	1	0	0	1 (5%)
Dyspnea	1	0	0	1 (5%)
Gastric ulcer	0	1	0	1 (5%)
Hematuria	1	0	0	1 (5%)
Hypophosphatemia	1	0	0	1 (5%)
Hypothyroidism	1	0	0	1 (5%)
Neutrophil count decreased	1	0	0	1 (5%)
Total:	123	5	0	

*****The values in parentheses represent the percentage of patients experiencing the indicated adverse event.

Note: Two patients developed second malignancies outside the protocol mandated adverse event-monitoring period (30 days after the last dose of selumetinib). One patient who received RAI on study was diagnosed with myelodysplastic syndrome more than 51 weeks after selumetinib, which then transformed to acute leukemia (please see the "Safety" subsection of the Results for more details). Another patient who did not receive RAI on study was diagnosed with chronic lymphocytic leukemia (CLL) 90 weeks after selumetinib. Both of these adverse events were unrelated to selumetinib.