Exceptional Response to AKT inhibition in breast cancer patients with germline *PTEN* mutations

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Introduction

Cowden syndrome is an autosomal dominant genetic disease with an estimated incidence of 1 in 200,000. Affected individuals develop multiple systemic hamartomas and have a cumulative lifetime risk of breast cancer of 85%[1]. Approximately 80% of patients with Cowden syndrome have a germline inactivating mutation in *PTEN* (10q23.3)[2]. *PTEN* acts as a tumor suppressor gene (TSG) via numerous mechanisms[3], one of which is antagonizing the PI3K/AKT/mTOR signaling pathway by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 functions as a secondary messenger in the PI3K pathway that binds and activates proteins that have a pleckstrin homology domain, such as AKT1, and triggers their activation and localization to the plasma membrane promoting cellular proliferation and survival[4].

Germline *PTEN* loss of function mutations may result in dominant AKT activation as a driving oncogenic event in Cowden-related breast tumours[5]. Pre-clinical evidence suggests that cancers with AKT activation have increased sensitivity to AKT inhibition[6]. Preliminary clinical evidence is derived from phase I and II trials in patients with breast cancers bearing somatic mutations in the PI3K-PTEN-AKT pathway[7-11].

Capivasertib (AZD5363, AstraZeneca) is a potent and selective oral inhibitor of all 3 isoforms of the serine/threonine kinase AKT (AKT1, 2, 3) with pre-clinical evidence of efficacy as monotherapy or in combination with cytotoxic and targeted therapies[12-15]. Despite the encouraging progression-free survival observed with capivasertib monotherapy in heavily pretreated patients with *AKT1* E17K-mutant breast and gynecologic cancers[9], RECIST response rates in phase 1 studies only reached 22% [8,9,16] (Table 1). Since PTEN loss activates AKT1, we hypothesized that tumors from patients with Cowden syndrome could be sensitive to this drug family.

Case 1 – SAFIR02 Trial

A 50-year-old woman with a family history of Cowden syndrome was diagnosed with a T3N3 breast cancer, estrogen (ER) and progesterone (PR) receptor-negative, HER2-negative grade III invasive carcinoma of no special type (NST). The patient received 6 cycles of neo-adjuvant EC-T (cyclophosphamide 600 mg/m^2, epirubicin 75 mg/m^2, docetaxel 100 mg/m^2) prior to a mastectomy with left axillary lymph node dissection (revealing residual disease in 10 of 18 lymph nodes) and adjuvant radiotherapy.

Eight months later the patient relapsed with cutaneous disease and thoracic nodal involvement. After enrolling in SAFIR02 (NCT02299999), targeted panel sequencing (Ion Torrent PGM) of a fresh tumor biopsy revealed the presence of a heterozygous germline *PTEN* mutation (c.389G>A, p.R130Q, SNP rs121909229) alongside other variants (Figure 1A and Supplementary Table S1). Immunohistochemistry revealed lack of PTEN expression in the tumor (Figure 1B). The patient received six cycles of paclitaxel (90mg/m^2 days 1, 8, and 15 of a 28 day cycle) with bevacizumab (10 mg/m^2 on days 1 and 15), and carboplatin (AUC2, days 1, 8, and 15 of a 28 day cycle, ceased after 6 weeks).

Upon completion of chemotherapy, tumor evaluation demonstrated a partial response (RECIST 1.1) with 60% reduction of target lesions (Figure 1C). In the context of SAFIR02, the patient was randomized to maintenance targeted therapy, and received capivasertib 480 mg BID 4 days on 3 days off. This treatment was well tolerated with no grade 2 or above toxicities. After 3-months of capivasertib monotherapy, a complete response was observed, which was maintained for 12 months before the patient progressed on capivasertib.

Case 2 – BEECH Trial

In March 2010 a 37-year-old woman with known Cowden syndrome and a history of a neck arteriovenous malformation, multinodular goiter and rectal hamartomatous polyps was diagnosed with bilateral breast cancer. On the right, she presented with a T3, ER- and PR-positive, HER2-negative grade II invasive carcinoma NST with 20 out of 23 involved lymph nodes. On the left, she presented with a 4mm grade II invasive carcinoma NST, strongly ER-positive and HER2-negative. Following a bilateral mastectomy, exome sequencing (Hiseq2500) of the right breast cancer and germline DNA revealed the germline *PTEN* mutation (c.T68G:p.L23X), and a 'second hit' somatic stop-gain *PTEN* mutation (exon 1, c.T264A:p.Y88X) with an AF of 25.5%, alongside other variants (Figure 2A and Supplementary Table S1). PTEN immunohistochemistry revealed reduced PTEN staining in the tumor (Figure 2B). Post-operative staging revealed metastatic disease, with mediastinal lymph nodes and lung metastases. In May 2010 the patient received 6 cycles of 3-weekly FEC chemotherapy (fluorouracil 600 mg/m^2, epirubicin 75 mg/m^2, cyclophosphamide 600 mg/m^2) before starting maintenance tamoxifen. In October 2011, she had a bilateral salpingo-oophorectomy.

After 28-month progression-free period, the patient was found to have new liver metastases. In November 2012 she enrolled in the phase 1/2 BEECH study (NCT01625286) and was assigned to receive paclitaxel plus capivasertib (Part A, Schedule 2). She received eight cycles of paclitaxel (90mg/m2 days 1, 8, and 15 of a 28-day cycle) combined with capivasertib (360mg BID on days 2-5, 9-12, 16-18 of each 28-day cycle). In June 2013, a CT showed a complete response of the liver metastases. The patient continued maintenance capivasertib alone with no grade 2 or above toxicities. She had a confirmed maintained response in May 2014 until progression in June 2014, giving a PFS of 19 months, and a

maintained complete response of 12 months on capivasertib alone (Figure 2C). Plasma ctDNA analysis from baseline and progression time points during the BEECH study showed no major changes (Supplementary Table S1).

Discussion

The AKT inhibitor capivasertib has been examined in early phase trials (Table 1) and no complete responses have been noted, yet both patients with Cowden syndrome presented here had durable complete responses to capivasertib. Such outlier sensitivity likely reflects the germline, and therefore fundamentally clonal, nature of *PTEN* alteration. *In vivo* mice models with *PTEN* homozygous deletion have shown dramatic regression of the Cowden phenotype features of trichilemmomas on treatment with mTOR (downstream from AKT) inhibitor rapamycin[17]. In humans, Hyman *et al* demonstrated that tumor response to targeted treatment with capivasertib was proportional to *AKT1* mutation clonality[9]. Furthermore, three case reports in pediatric patients and a pilot study (n=18) in adults with *PTEN* aberrations have shown regression of phenotypic changes associated with *PTEN* loss after treatment with mTOR inhibitor sirolimus[18-21], demonstrating sensitivity of even non-malignant cells carrying a germline *PTEN* mutation to PI3K-pathway inhibition. Similarly to the cases presented here, no severe toxicity was noted in the pilot study[21], with just one patient experiencing a Grade 3 AE (hypophosphatemia/hypercholesterolaemia).

In recent years drugs targeting the PI3K/AKT/mTOR pathway have been developed. Trials have attempted to identify molecular predictors of response by identifying alterations in the PI3K/AKT/mTOR pathway (Supplementary Table S2). Tumor *PIK3CA* mutations are not clear predictors of response to mTOR inhibition. In contrast, AKT inhibitors in combination with paclitaxel have been shown to be more active in patients with triple negative breast

cancers harboring a *PIK3CA/PTEN/AKT1* pathway alteration.[7,22] Similarly, PI3kinase inhibitors have demonstrated activity in patients with *PIK3CA* mutations[23,24].

Of note in Case 2 is the presence of the second-hit *PTEN* stop-gain mutation Y88X present with an AF of 25.5%. However, the presence of a *TP53* mutation at an AF of 51.9% indicates that this 'second-hit' mutation is subclonal rather than a truncal driver mutation. Conversely Case 1 does not have a *PTEN* second-hit mutation or loss of heterozygosity (LOH) yet demonstrates lack of PTEN expression. Low tumor purity can make LOH difficult to detect, and whilst the estimated purity was 40%, the true purity may have been lower. Explanations for the PTEN phenotype in Case 1 include undetected LOH, PTEN promotor hypermethylation[25,26], complex PTEN genomic rearrangements[27] and post-translational modification[28].

Contrary to Knudson's 'two-hit' model of tumorigenesis in TSGs[29], *PTEN* aberrations appear to be pro-tumorigenic in the absence of a 'second hit'. In 2010, Alimonti *et al* demonstrated that PTEN hypermorphic mice (with 80% of normal PTEN protein level) had a greater propensity to tumorigenesis than mice with two functional alleles, but were less tumorigenic than *PTEN* heterozygous mice[30], supporting a haploinsuffiency model of tumorigenesis in *PTEN* aberrations.

A later *in vivo* study of *PTEN* knock-in mice models suggested that the conformation of PTEN underlies the dominant-negative behavior of *PTEN* heterozygous mutants. Papa *et al*[31] demonstrated that PTEN is catalytically active in PIP3 dephosphorylation and subsequent downstream PI3K/AKT/mTOR pathway regulation, after dimerization. Significantly, mutant PTEN protein was able to dimerize with wild type PTEN but the resultant hetero-dimers were less able to hydrolyze PIP3. Moreover, mutant PTEN outcompeted and displaced wild type

PTEN protein in dimerization and membrane localization. This supports the rationale that *PTEN* heterozygous mutants act in a dominant-negative manner to promote tumorigenesis.

The cases presented here were treated with combination chemotherapy and capivasertib followed by capivasertib monotherapy. The patient in Case 1 had previously demonstrated tumor resistance to taxane therapy, with residual disease following neo-adjuvant chemotherapy and a short progression free survival after treatment for primary breast cancer. The second patient achieved a complete response with the combination of paclitaxel and capivasertib, and maintained this complete response for a period of 12 months on capivasertib alone, suggesting that capivasertib was highly active in this patient.

In summary, these two breast cancer patients with different germline *PTEN* mutations both showed a dramatic response to capivasertib superior to that seen in early trials of the drug. The excellent response in these two patients, despite their differing histology, is a promising indication that targeted AKT therapy is an effective approach in patients with germline *PTEN* mutations.

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Figure and Table Legends

Table 1. Summary of main phase I clinical trials with capivasertib monotherapy.

Figure 1 Exceptional response in patient with germline *PTEN* R130Q mutation.

A. Germline *PTEN* mutation c.389G>A, pR130Q. Illustration from https://proteinpaint.stjude.org. B. Immunohistochemistry demonstrating (i) absent PTEN staining in the tumor, (ii) cytoplasmic and membranous expression of pAKT in the 40% of tumor cells. C. CT scans during the patient's time on capivasertib, white arrows indicating axillary disease. (i), September 2015, baseline CT showing two areas of axillary lymphadenopathy. (ii) January 2016, CT scans demonstrating partial response following 4 months of Carboplatin-Paclitaxel-Bevacizumab chemotherapy. (iii) December 2016, CT scans following 11 months of capivasertib monotherapy demonstrating persistent complete response before progression in February 2017.

Figure 2. Exceptional response in patient with germline *PTEN* L23X mutation.

A. Germline *PTEN* mutation c.T68G:p.L23X and "second hit" *PTEN* mutation c.T264A:p.Y88X. Illustration from https://proteinpaint.stjude.org. B. PTEN immunohistochemistry from control tissue (i), and non-cancerous (ii) and tumor-containing lymph node (iii and iv). The control tissue shows mainly cytoplasmic expression of PTEN. The non-cancerous lymph node and the residual lymphatic tissue in the lymph node metastasis (*) also show a PTEN expression comparable with the control. The tumor cells in the lymph node metastasis display weaker PTEN staining, which is mainly nucleolar. C. CT scans during the patient's time on capivasertib, white arrows indicating disease in the liver. (i) October 2012, baseline CT demonstrating a liver deposit. (ii) January 2013, CT following

2 cycles of paclitaxel and capivasertib. (iii) CT demonstrating continued complete response on maintenance capivasertib 7 months after cessation of paclitaxel. (iv) May 2014, final CT before progression June 2014.

Table 1. Summary of main phase I clinical trials with capivasertib monotherapy.

Trial	Phase	Number of Patients	Key Eligibility	Treatment Regimen	Results
Banerji *et al*, 2018[8] NCT01226316	I	Part A and B: 90 Part C: 59	Parts A and B: Advanced solid malignancy (Western dose finding) Part C: ER+ or HER2+ breast cancer or gynecological cancer with *PIK3CA* mutation	Part A and B: dose escalation and expansion at multiple dosing schedules[1] Part C: dose expansion of capivasertib at monotherapy RP2D (480mg BD for 4 days on, 3 days off)	Part A and B: 1 confirmed PR in *PIK3CA* mutant cervical cancer patient. 27 (30%) and 6 (7%) of patients had SD for ≥6 and ≥12 weeks, respectively. Part C: Of 54 included in the RECIST assessment, 3 (5.6%) had a confirmed PR, of whom 1 of 28 (4%) in the ER+ breast cancer cohort and 2 of 26 (8%) in the gynecological cancer cohort
Hyman *et al*, 2017[9] NCT01226316	I	59 (Evaluable =58)	Part D: ER+ or HER2+ breast cancer, gynecological cancer, all other solid tumors with *AKT1* mutation (E17K, n = 52; non-E17K, n = 5; *AKT1* mutation not detected, n = 1).	Part D: dose expansion at monotherapy RP2D 480mg BD for 4 days on, 3 days off.	Confirmed PR in 10 of 58 (17.2%), of whom 4 of 20 (20%) patients in the ER+ breast cancers cohort, 4 of 18 (22.2%) patients in the gynecological cancers cohort, and 2 of 20 (10.0%) patients in the other solid tumor cohorts (triple-negative breast cancer and lung adenocarcinoma, n = 1 each).
Tamura *et al*, 2016[16] NCT01353781	I	41	Advanced solid malignancy (Japanese dose finding)	Dose escalation at multiple dosing schedules[2]	Of 37 evaluable patients, 2 (5.4%) had confirmed PR, and 10 (27%) had SD for ≥6 weeks.

Abbreviations: ER+, estrogen receptor positive; HER2+, HER2 positive; HER2-, HER2 negative; RP2D, recommended phase II dose; PR, partial response; SD, stable disease.

Figure 1. Exceptional response in patient with germline *PTEN* R130Q mutation.

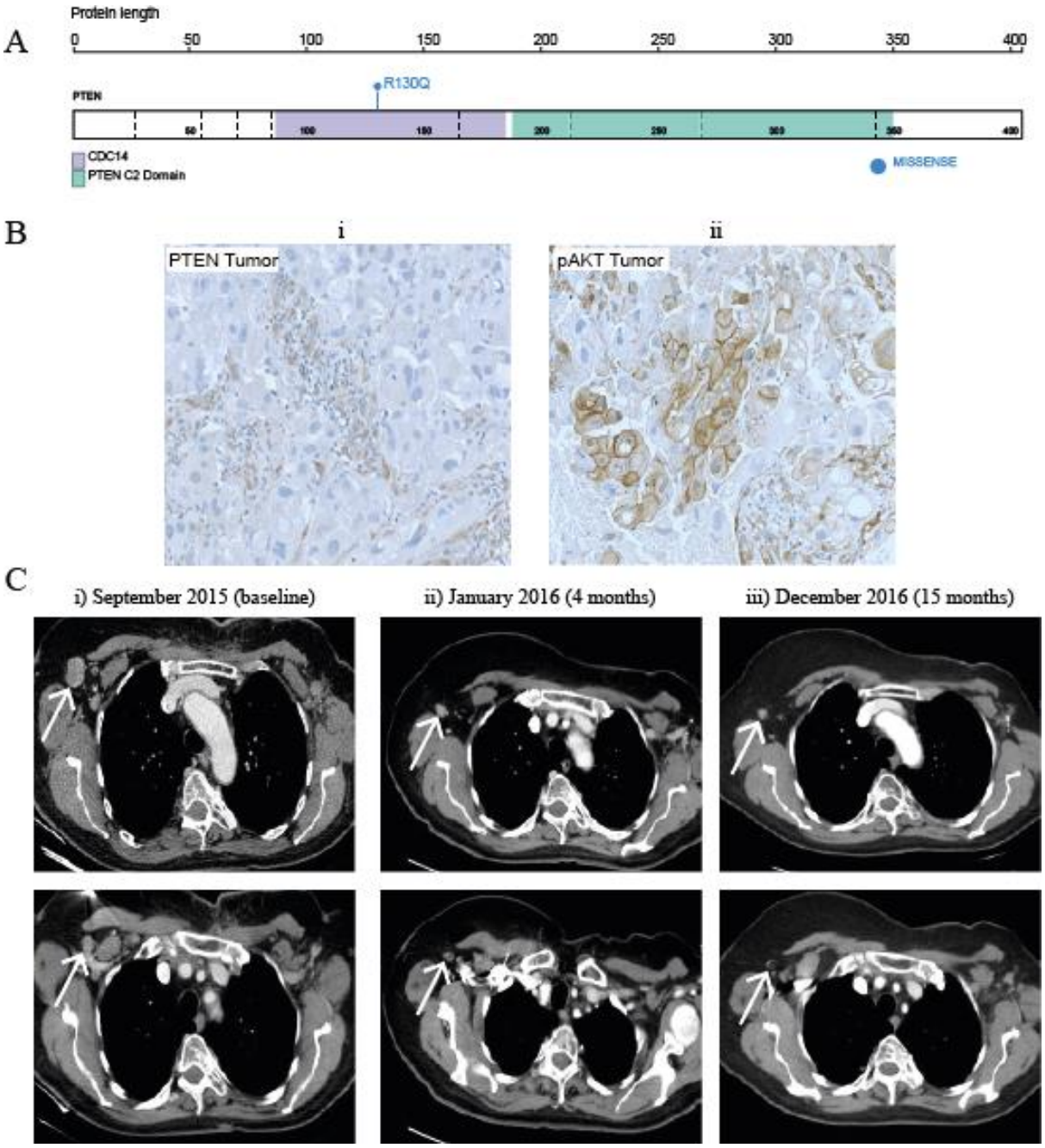
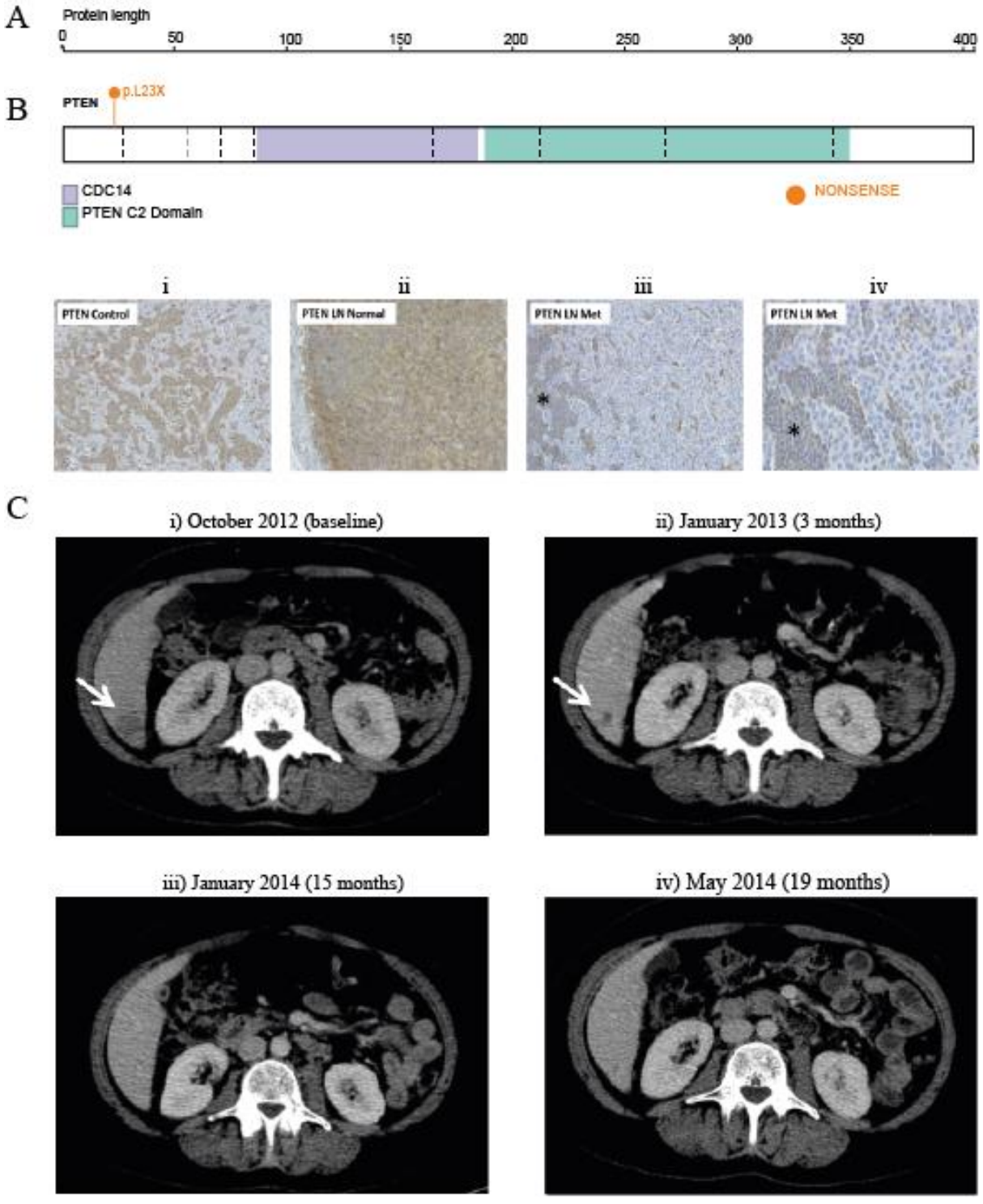


Figure 2. Exceptional response in patient with germline *PTEN* L23X mutation.


A
Protein length
0
50
100
150
200
250
300
350
400
B
PTEN
p.L23X
CDC14
PTEN C2 Domain
NONSENSE
i
ii
iii
iv
PTEN Control
PTEN LN Normal
PTEN LN Met
PTEN LN Met
C
i) October 2012 (baseline)
ii) January 2013 (3 months)
iii) January 2014 (15 months)
iv) May 2014 (19 months)