Supplemental Information:
Text

The proband: The proband was a 29 year old female at the time of presentation. She was referred to UT Southwestern Medical Center after a left upper lobe mass was discovered during work up for syncope. She had a past history of pulmonary tuberculosis and hypothyroidism. PET/CT scan revealed a fludeoxyglucose avid 4.4 cm L upper lobe mass, a fludeoxyglucose avid hilar node, multiple bilateral subcentimeter ground glass nodules, as well as calcified hilar lymph nodes (Supplemental Fig. 1). Transbronchial biopsy of the left upper lobe mass indicated a poorly differentiated adenocarcinoma, TTF1 and CK7 positive. Because of the possibility of metastatic disease, wedge biopsies of the right upper and lower lobe were performed. Subsequently a robotic left upper lobectomy was performed for a T2aN0M0 poorly differentiated adenocarcinoma. The wedge biopsies of the right lung contained multiple lesions with a spectrum of pathologies (Supplemental Fig. 2) ranging from atypical adenomatous hyperplasias to adenocarcinomas in situ and minimally invasive adenocarcinoma.[1] She received
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DNA was isolated from formalin--fixed paraffin--embedded tissue and sequenced in the forward
and reverse direction. All sequences were read manually. Molecular testing of the LUL tumor demonstrated no ALK gene rearrangement, and absence of mutations in BRAF and KRAS genes. Analysis of *EGFR* exons 18--21 by Sanger sequencing revealed an L858R mutation in exon 21 (minor peak in comparison to the wild type peak), and a T790M mutation in exon 20 at coding nucleotide 2369 (equivalent in height to the wild type peak) (Fig. 1). The mutations were confirmed by sequencing in both directions. Mutation analysis of her blood mononuclear cells indicated a T790M mutation, with equivalent heights of the mutant and wild type peaks. These findings confirmed the presence of a germline T790M mutation.
History and mutation status of Proband's family: Information (relationship, cancer status, age, available CT scans, gender, and smoking status) was obtained for five generations Fig. 2, Supplemental Table 1). Mutation testing was performed on three generations. Eight of 17 family members tested were positive for the mutation, including the proband's mother and brother (her sole full sibling). It was determined that the proband inherited the mutation from her mother. Eight of 14 maternally related family members tested were mutation positive (57.1%), consistent for Mendelian inheritance of an autosomal gene. In addition, four more family members are obligate carriers for the T790M mutation based on the family pedigree, for a total of 12 mutation carriers. Four of these 12 had lung cancer. In addition, there was a never smoker that was reported to have died from bladder cancer at the age of 60. She also had lung cancer, but medical records were not available to confirm if the lung cancer was a second primary or a metastasis. This individual was not included as a lung cancer case in our analyses. Another mutation carrier had a lung carcinoid at the age of 31. Genetic testing was not performed on two deceased family members who were never smokers with lung cancer. For our analysis we have assumed that these individuals were carriers for the familial T790M mutation. Given their a priori risks of a germline mutation of 50% and 25%, it is highly unlikely that they were sporadic cases of lung cancer in never smokers. CT scans were available on 5 mutation carriers. In all the unaffected carriers one or more subcentimeter solid or ground glass nodules of uncertain etiology were identified (Supplemental Table 2). As ground glass vs solid morphology on CT scans generally correspond to preinvasive (atypical adenomatous hyperplasia or adenocarcinoma in situ), minimally invasive

adenocarcinomas or lepidic adenocarcinoma

pattern vs. invasive adenocarcinoma patterns by pathology respectively, these CT findings suggest these patients also had a similar spectrum of preinvasive, minimally invasive and overtly invasive adenocarcinomas. Of interest the CT scan of the 80 year old grandfather of the proband, a lifetime heavy smoker, had a solitary small solid nodule along with severe emphysematous changes (which was never biopsied).

Lung cancer arising in T790M germline carriers: From the five reports in the literature and our Proband's family pedigree, we identified 19 cases of lung cancer arising in known or assumed carriers (Table 1). The analysis is complicated because of two factors:

The report by Oxnard et al [2] is complicated because two of their five cases had been previously reported by Girard et al,[3] and that some non--essential patient details were altered or hidden for confidentiality reasons. However the two previously reported cases are not identified. After discussions with Geoffrey Oxnard (personal communication) and a reconstruction of the cases to the best of our ability, we believe we have correctly identified the non--overlapping cases (Table 1). However, some details such as age and smoking history can only be ascertained for the group.

Another rare germline mutation of the *EGFR* gene, V843I in exon 21[5-7] is associated with clinical and pathological features similar to those of germline T790M mutations. The similarities include preferences for female gender, adenocarcinoma histology, multiple lesions and resistance to TKI therapy. Germline V843I mutations may occur with or without a second activating *EGFR* mutation, appears to be inherited dominantly and may occur in smokers or never smokers. Unlike T790M, two of the three reported families are of East-Asian ethnicity.

Discussion on report of germline T790M mutations reported by Tibaldi et al.

The report by Tibaldi et al describes lung cancers arising in two Italian never smoking sisters with germline T790M mutations.[4] However the report contains multiple contradictory or incorrect statements or illustrations. The proband's age is reported as 72 years in the text and as 73 years in the pedigree, and her tumor pathology is reported as being adenocarcinoma in the text and as squamous cell in the pedigree. We chose to interpret the tumor as an adenocarcinoma, as squamous cell lung carcinomas are exceedingly rare in lifetime never smokers.[8] The positions of

the germline mutations are indicated by arrows, but the arrows appear to point to nucleotide 2366 instead of the correct site, nucleotide 2369. Although the peaks are small, there appears to be a single thymidine peak at nucleotide 2369 in the electropherograms of blood samples of both sisters, which would indicate that both sisters have homozygous germline T790M mutations. This is highly unusual and has never been reported elsewhere (and is not mentioned or discussed in their report). As described in the next section, the estimated prevalence of the heterozygous mutation is predicted to be less than 1 in 7500. Thus the odds of both parents being heterozygous are less than 1 in 56 million, and the odds that two siblings are homozygous carriers are less than 1 in 896 million.

Supplemental References

1. Travis WD, Brambilla E, Noguchi M, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma. *J Thorac Oncol* 2011; **6**(2): 244--85.
2. Oxnard GR, Miller VA, Robson ME, et al. Screening for Germline EGFR T790M Mutations Through Lung Cancer Genotyping. *J Thorac Oncol* 2012; **7**(6): 1049--52.
3. Girard N, Lou E, Azzoli CG, et al. Analysis of genetic variants in never--smokers with lung cancer facilitated by an Internet--based blood collection protocol: a preliminary report. *Clin Cancer Res* 2010; **16**(2): 755--63.
4. Tibaldi C, Giovannetti E, Vasile E, et al. Inherited germline T790M mutation and somatic epidermal growth factor receptor mutations in non--small cell lung cancer patients. *J Thorac Oncol* 2011; **6**(2): 395--6.
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6. Ohtsuka K, Ohnishi H, Kurai D, et al. Familial lung adenocarcinoma caused by the EGFR V843I germ--line mutation. *J Clin Oncol* 2011; **29**(8): e191--2.
7. Demierre N, Zoete V, Michielin O, et al. A dramatic lung cancer course in a patient with a rare EGFR germline mutation exon 21 V843I: Is EGFR TKI resistance predictable? *Lung cancer (Amsterdam, Netherlands)* 2013.
8. Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012; **489**(7417): 519--25.

Supplementary Table 1. Summary of information about proband's family. Generations are indicated by Roman numerals. The proband is subject V--3. Also see family tree (Fig. 1).

Individual	Sex	Age	Lung Cancer	Never Smoke	Smoker	T790M status	Risk of Mutation
I--1	M	91			X	N/A	50%
I--2	F	72		X		N/A	50%
II--1	F	70s		X		Obligate carrier	
II--2	F	67	X	X		N/A	50%
II--3	M	78			X	N/A	50%
II--4	M	84			X	N/A	50%
II--5	F	89		X		N/A	50%
II--6	M	70		X		Obligate carrier	
III--2	F	81	X	X		Obligate carrier	
III--3	F	60		X		N/A	50%
III--4	F	76		X		N/A	50%
III--5	F	80		X		N/A	50%
III--6	M	56	X	X		N/A	25%
III--7	F	55		Unknown	Unknown	N/A	50%
III--8	F	72		X		negative	
III--9	F	77		X		positive	
III--10	M	77			X	positive	
III--11	F	67		X		negative	
III--12	M	80			X	positive	
III--13	F	75			X	negative	Tested for lineage
IV--1	M	63			X	positive	
IV--2	F	55		X		N/A	50%
IV--3	M	65		Unknown	Unknown	N/A	25%
IV--4	F	60		Unknown	Unknown	N/A	25%
IV--5	M	67		X		negative	
IV--6	M	66			X	negative	
IV--7	M	57			X	N/A	50%
IV--8	M	56			X	N/A	50%
IV--9	M	40		X		N/A	50%
IV--10	M	56		X		N/A	50%
IV--11	M	54		X		N/A	50%
IV--12	M	57		X		negative	Tested for lineage
IV--13	F	57		X		positive	
IV--15	F	56		X		negative	
IV--16	M	31		X		N/A	50%
V--1	M	33			X	positive	

V-2	M	28		X		negative	50%
V-3	F	29	X		X	positive	
V-4	M	26			X	positive	
V-5	M	35			X	negative	
V-6	F	33		X		Obligate negative	
V-7	F	28		X		Obligate negative	
V-8	F	21		X		Obligate negative	
V-9	M	38		X		N/A	25%
V-10	F	33		X		N/A	25%

(Supplemental Table 1 continued)

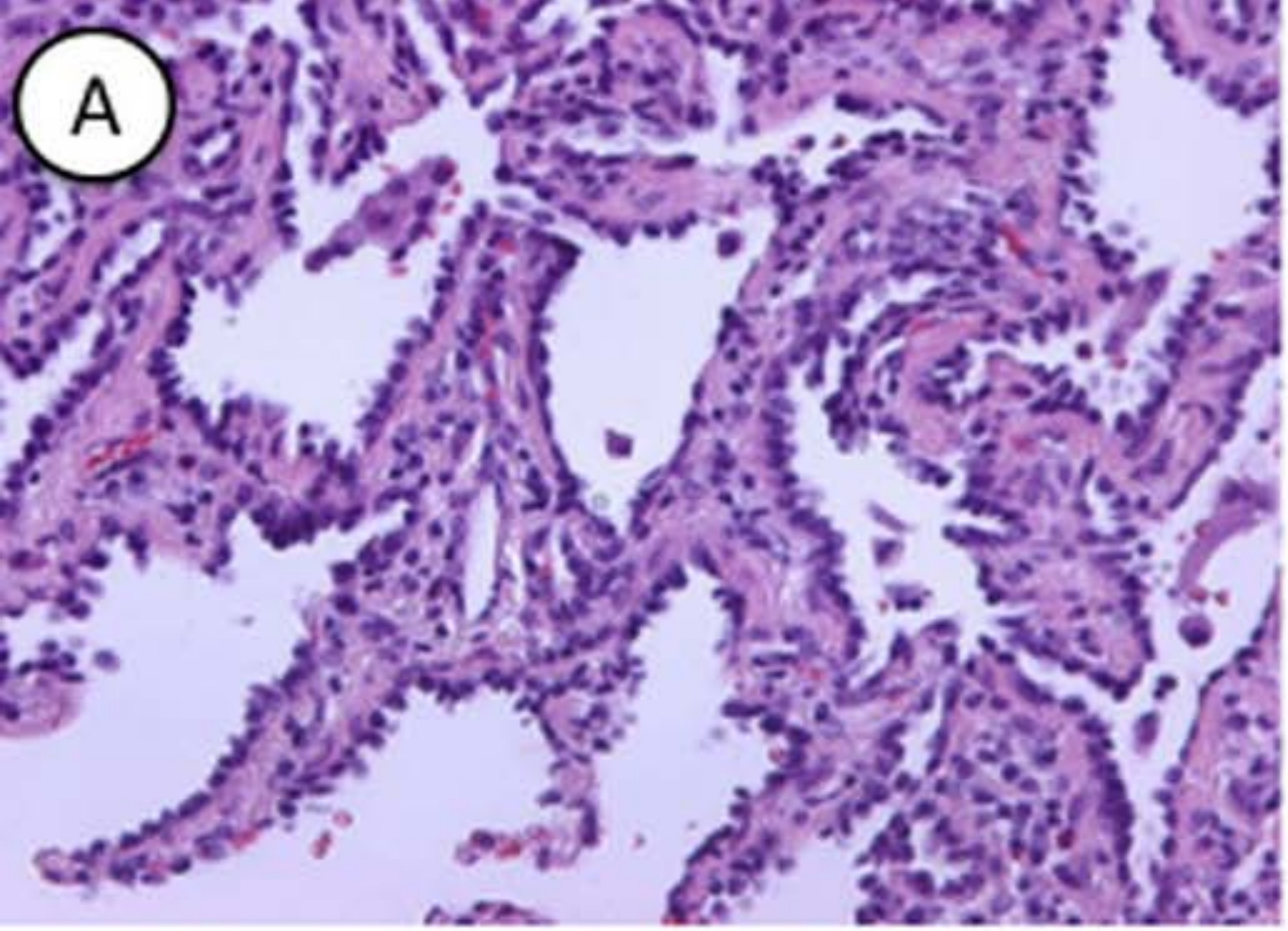
Supplemental Table 2. Chest CT scan findings of five carriers of the germline T790M family (also see Supplemental Table 1 and Fig. 2).

- Five unaffected mutation carriers (as indicated on the Family pedigree) had one or more low dose CT scans:
- IV--13 (Proband's mother -- never smoker) multiple bilateral small nodules including ground glass opacities, stable over the course of one year
- V--4 (Proband's brother -- light smoker) multiple bilateral small nodules including ground glass opacities, stable over the course of one year
- IV--1 (distant relative -- heavy smoker) three subcentimeter nodules.
- V--1 (distant relative -- light cigar smoker--) three subcentimeter ground glass nodules.
- III--12 (Proband's grandfather) Emphysematous changes and single subcentimeter solid nodule.

Legends for Supplemental Figures

Supplemental Figure 1. Chest CT scans of proband prior to surgery. A large 4 cm mass is present in the le= upper lobe (A). A small subcentimeter ground glass opacity is also present in the le= lower lobe (B).

Supplemental Figure 2. Pathology of resected lung lesions (photomicrographs of H. and E. stained sections). Panels A and B are independent atypical adenomatous hyperplasias from the right lung. Panel C illustrates a microinvasive adenocarcinoma from the right upper lobe. Panel D illustrates the invasive adenocarcinoma resected from the le= upper lobe. The tumor stained positively for TTF1 (NKX2-1) and cytokeratin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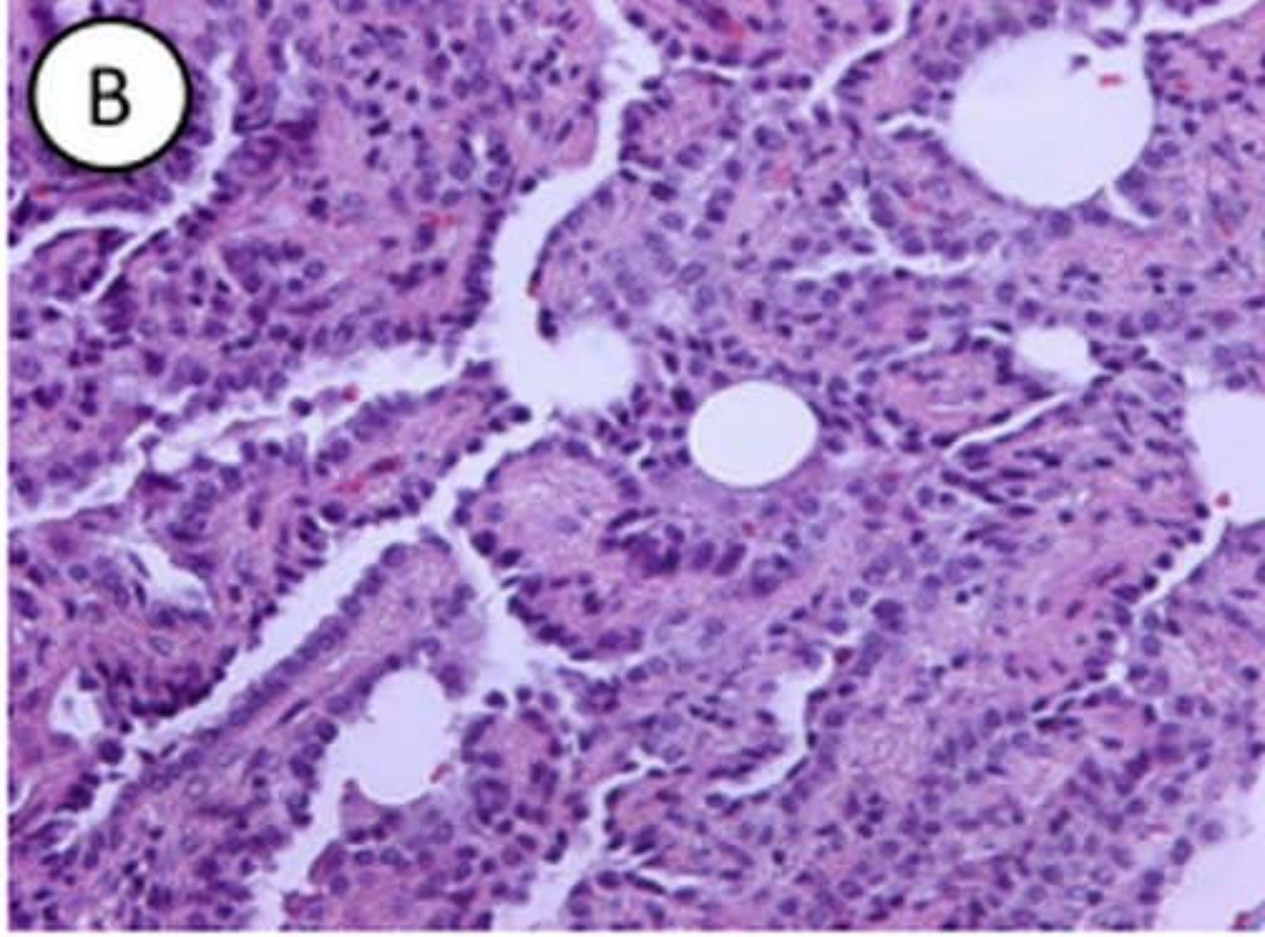
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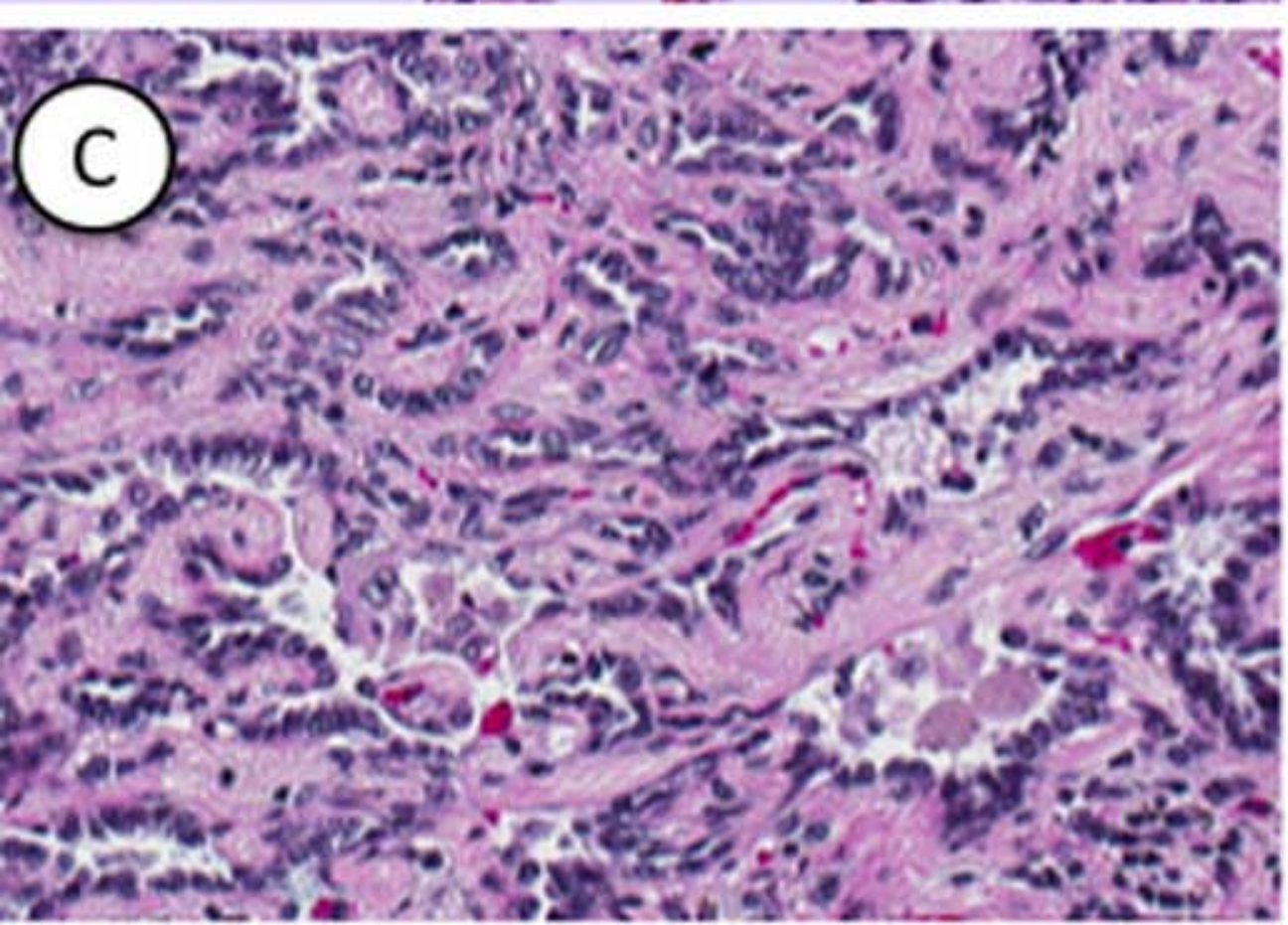
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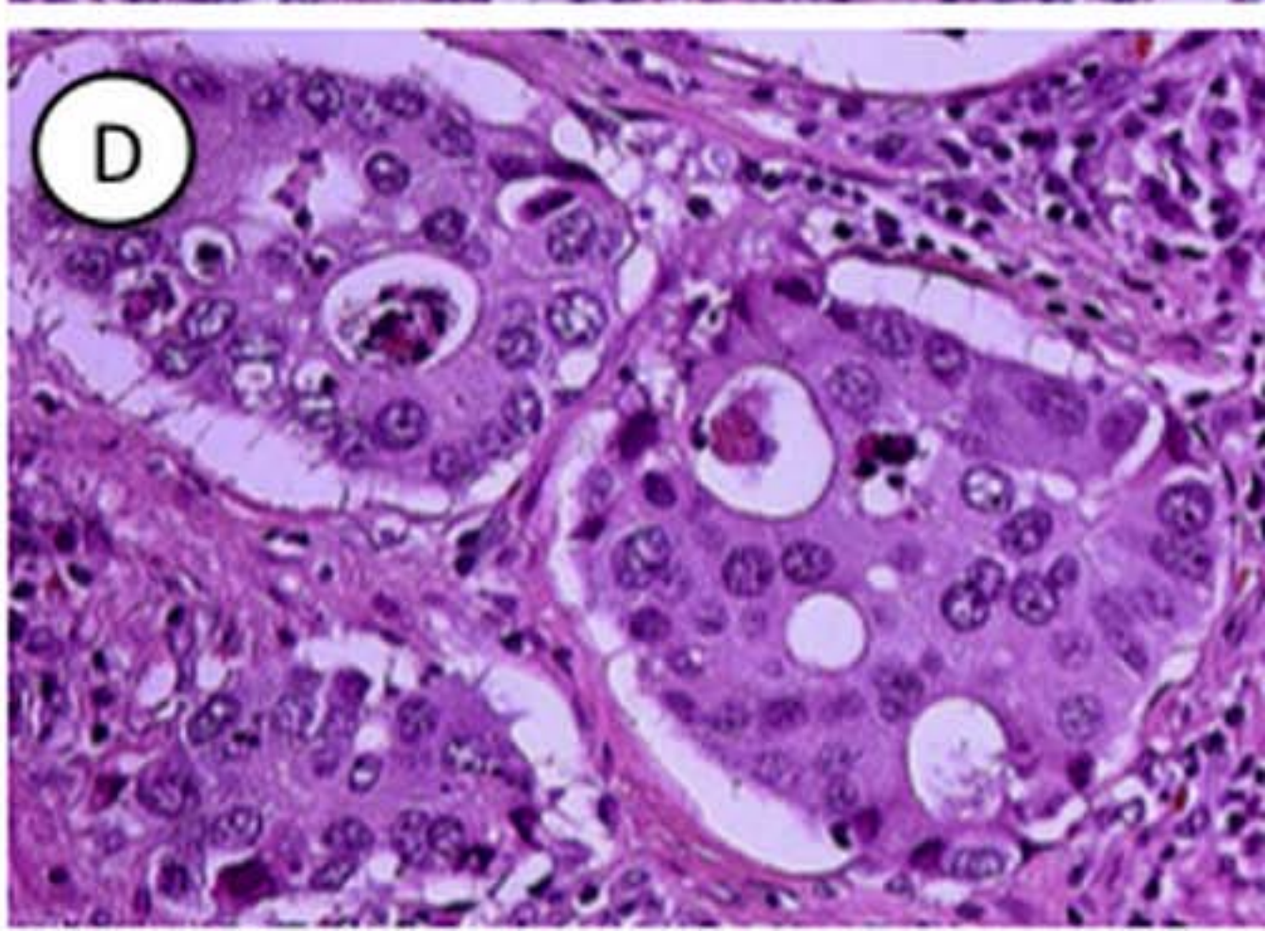
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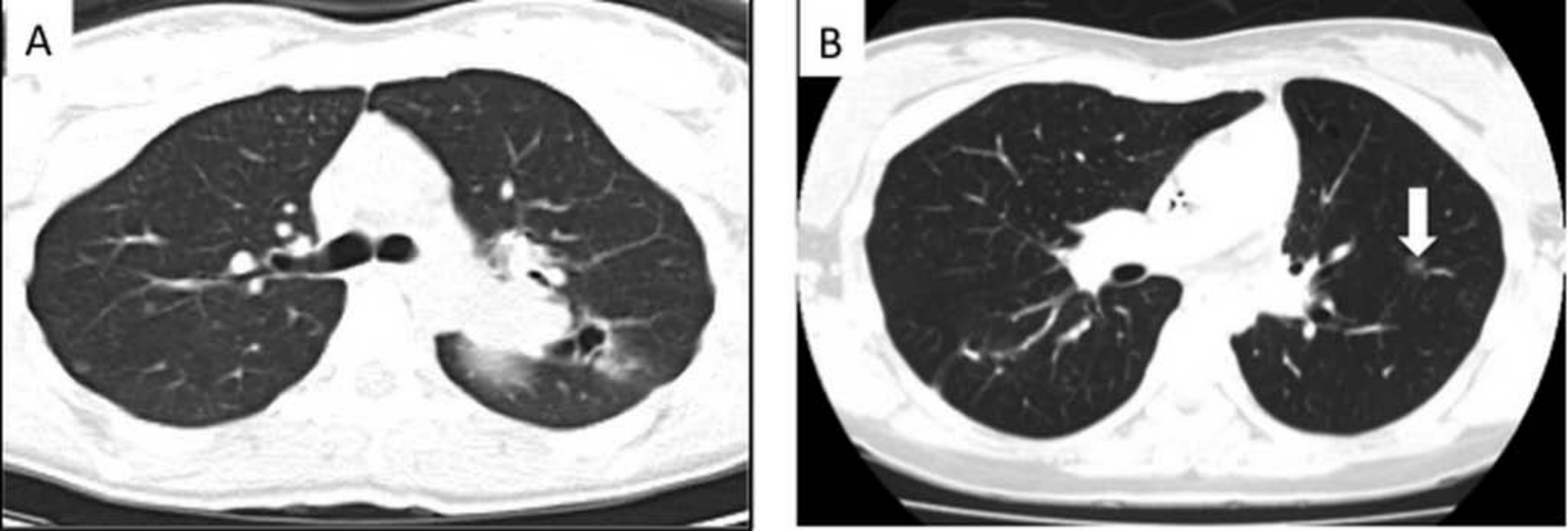
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