PIK3CA mutation in gastric cancer and the role of microsatellite instability status in mutations of exons 9 and 20 of the *PIK3CA* gene

Karol Polom[1,6,A-F], Daniele Marrelli[1,A–F], Giandomenico Roviello[2,3,A–F], Valeria Pascale[1,B,E,F], Costantino Voglino[1,B,C,E,F], Carla Vindigni[4,B,E,F], Daniele Generali[5,C,E,F], Franco Roviello[1,A–F]

[1] Unit of General Surgery and Surgical Oncology, University of Siena, Italy
[2] Department of Oncology, Medical Oncology Unit, San Donato Hospital, Arezzo, Italy
[3] Department of Medical, Surgery and Health Sciences, University of Trieste, Italy
[4] Department of Pathology, Hospital Department of University of Siena, Italy
[5] Department of Medical, Surgery and Health Sciences, University of Trieste, Italy
[6] Department of Surgical Oncology, Medical University of Gdansk, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899-5276 (print), ISSN 2451-2680 (online) *Adv Clin Exp Med*. 2018;27(7):963–969

Address for correspondence
Karol Polom,
E-mail: polom.karol@gmail.com

Funding sources
Istituto Toscano Tumori (ITT) grant entitled "Gene expression profiles and therapy of gastric cancer" – (grant No. ITT-2007) European Union's Seventh Framework Programme (FP7), GastricGlyco-Explorer under grant agreement No. [316929] (Karol Polom, Franco Roviello).

Conflict of interest
None declared

Received on September 21, 2016
Reviewed on February 13, 2017
Accepted on April 27, 2017

Abstract

Background. A better understanding of molecular gastric cancer (GC) entities may help in tailored treatments of that neoplasm. The *PIK3CA* mutation is one of the most important in many cancers.

Objectives. We performed a comparison of clinical and pathological data of the *PIK3CA* mutation in GC patients.

Material and methods. The analysis was done on 472 patients operated on in 1 center. Polymerase chain reaction (PCR) was used for the screening of *PIK3CA* (exon 9 and 20). For microsatellite instability (MSI) we used 5 quasi-monomorphic mononucleotide repeats — BAT-26, BAT-25, NR-24, NR-21, and NR-27. The clinical and pathological data was analyzed.

Results. *PIK3CA* mutation was observed in 10 out of 472 GC patients (2.1%). Nine out of 10 were MSI (9 of 111 MSI patients – 8.1%). Half of the 10 patients had mutations in exon 9 and the other half in exon 20. A majority of patients with the *PIK3CA* mutation had MSI ($p < 0.001$). The 5-year survival of MSI patients with the *PIK3CA* mutation was 40% and without the mutation, 70.4% ($p = 0.309$). For patients with the mutation in exon 9, the 5-year survival was 0%, and for those with the mutation in exon 20, 80% ($p = 0.031$). The Cox proportional hazards regression analysis did not show that *PIK3CA* is statistically correlated with a worse overall survival.

Conclusions. *PIK3CA* mutation in GC is a rare finding. It is strongly associated with the MSI molecular subgroup, presenting a worse outcome than other MSI patients. A completely different outcome is associated with the mutation in exon 9 compared to the mutation in exon 20, with the latter being more favorable.

Key words: gastric cancer, *PIK3CA*, mutation, microsatellite instability, exon

DOI
10.17219/acem/70795

Introduction

Gastric cancer (GC) is a disease that is characterized by multiple molecular, genetic, and epigenetic events.[1] Mutation in a signaling pathway is one such event and the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of the rapamycin pathway (PI3K/AKT/mTOR pathway) is one example of the mutation mechanism.[2] A fundamental step in this pathway is the creation of phosphatidylinositol-3,4,5-triphosphatate (PIP3), catalyzed by PI3K. This pathway is important in the cancer-related functions of cell proliferation, catabolism, cell adhesion, apoptosis, and autophagy.[3] It also plays an important role in motility and glucose homeostasis.[4] The mutations of this pathway have frequently been seen in cancers such as ovarian, breast, thyroid, and cervical. Many studies have revealed that this pathway is not of highest importance in GC patients. The *PIK3CA* gene is located on chromosome 3p26.3.[5] The most common mutations are seen in exon 9 and 20, representing different hotspots of mutations.[4]

In the current literature, *PIK3CA* mutations in colorectal cancer (CRC) are associated with female gender, proximal position, well-differentiated tumors, and mucinous histology, but these findings are not consistent.[6] Some studies have shown a significant coexistence of *PIK3CA* and *KRAS* mutations, while other studies have failed to show such a coexistence. Other contradictory data presented a link between the *PIK3CA* mutation and MSI or CpG island methylator phenotype (CIMP).[6] Another inconclusive study showed worse outcome for early stage resectable disease, but other studies did not prove this finding.[6] Such conflicting findings can be explained by important differences between cancers that have the mutation in exon 9 vs those with the mutation in exon 20.[6,7] Exon 20 has a mutual relationship with *BRAF* mutation, CIMP high/low and MSI-H, and exon 9 is linked with KRAS mutations.[7] In the study by Mao et al., authors showed that mutations in exon 20 are associated with resistance to anti-EGFR antibody therapy. This was not seen for mutations in exon 9.[8] Tapia et al. reported that PI3K and AKT are overexpressed in GC with lymph node spread.[9]

New molecular classifications of gastric cancer have recently been proposed.[1,10] We are witnessing huge advances in our understanding of cancer from a molecular, immunological, diagnostic and even bioinformatical standpoint, all contributing to better tailored treatments for patients.[1,10–15] In both classifications, microsatellite instability (MSI) is a distinct molecular subgroup of GC. In the available studies, MSI is associated with older age, female gender, intestinal histotype, non-cardia tumors, lower number of metastatic lymph nodes, and better survival.[16–18] It seems that the MSI subgroup is not homogenous and other genetic and molecular factors may play an important role for these particular patients.

The aim of the study was to compare the clinical and pathological data of *PIK3CA* mutation in GC patients. We divided *PIK3CA* mutations into 2 categories based on the hotspot mutation sites at exon 9 and exon 20. In addition, we investigated the coexistence of this mutation with MSI status and *KRAS* mutations.

Material and methods

Patients

The analysis was performed on a group of 472 GC patients treated in the General Surgery and Surgical Oncology Department, University of Siena, Italy. We used tissue material stored in our biobank collected from patients who were operated on between 1990–2011. None of these patients received neoadjuvant treatment. We used tumoral and healthy tissues for comparative analysis. All samples were collected just after resection in the operating theatre.

PIK3CA sample preparation

Genomic DNA was extracted by tumoral and constitutional fresh frozen sample tissues using a standard protocol (Gentra Systems, Minneapolis, USA). The DNA concentration was calculated by spectrophotometry.

Polymerase chain reaction (PCR) is used for the screening of *PIK3CA* (exon 9 and 20).

To search for somatic alterations of the *PIK3CA* gene, exons 9 and 20 were sequenced according to the protocol described in detail by Velho et al.[19] PCR reactions were carried out in a volume of 20 µL containing 100 ng/µL genomic DNA template, 1X Reaction Buffer, 0.5 µM of each PCR primer, $MgCl_2$ 1.25 mM, 0.15 mM of each dNTPs, *Taq* polymerase 0.5 U/µL (Euroclone, Pero, Italy). The reactions were performed in programmable thermocyclers according to the standard protocol.

A 5 µL aliquot of each PCR reaction was run on a 2% agarose gel to confirm the size, quantity, and purity of each PCR product. The remaining 15 µL of PCR amplified bands were extracted from the gel with the Invisorb® Spin DNA Extraction Kit (Invitek, Stratec Biomedical Systems, Birkenfeld, Germany). Samples were then purified and 2 µL aliquot of purified PCR product was cycle sequenced using a BigDye Terminator Kit (Applied Biosystems, Foster City, USA) in a total volume of 20 µL. Samples were then purified and sequenced using an automated DNA sequencer ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Milan, Italy) according to the protocol of the manufacturer. Sequencing was performed in both strands. All sequence alterations in these genes were validated with a second independent PCR.

Pentaplex polymerase chain reaction and microsatellite analysis

A detailed description of MSI analysis was described in our previous paper.[18] In short, we used 5 quasi-monomorphic

mononucleotide repeats, namely, BAT-26, BAT-25, NR-24, NR-21, and NR-27. Following the definition of the National Cancer Institute workshop on MSI for cancer, we considered a tumor as MSI when 2 or more markers showed instability on 5 loci (MSI-H).[20]

A detailed description of the pathological, clinical, surgical and follow-up data was also given in our previous publication.[18]

Statistical analysis

Statistical analysis was performed with the χ^2 test or Fisher exact test to compare categorical variables. The Mann-Whitney U test was used to compare continuous variables not normally distributed. Cumulative survival was calculated by the life table method of Kaplan and Meier, and the log-rank test was used to distinguish significant differences. Statistical significance was determined at p-value < 0.05.

Survival curves estimated using the Kaplan-Meier method were compared using a log-rank test, considering death from cancer as the end-point (cancer-related survival).

The Kaplan-Meier estimation was used to plot survival curves, and log-rank tests were used to calculate the difference of overall survival (OS) between groups.

Multivariate Cox proportional hazard regression analysis was used to investigate independent prognostic factors for overall survival between groups. The variables including *PIK3CA* status, age, sex, tumor location, MSI status, Lauren histotype, type of resection, T, N, M status, and adjuvant therapy were used as covariates. Statistical analysis was done using commercially available statistical software (SPSS 20.0 for Windows SPSS Inc., Chicago, USA).

Results

PIK3CA mutation was observed in 10 of 472 GC patients (2.1%). Half of the 10 patients had mutations in exon 9 and the other half in exon 20. For exon 9 mutations, we found 2 mutations of *E542K*, 1 mutation of *E545K*, 1 of *N515S*, and 1 of *E545G*. All 5 patients with a mutation in exon 20 had mutation of *H1047R*.

Interestingly, 9 of 10 patients were also MSI positive. In 2 patients (20%), we observed *KRAS* mutation and *PIK3CA* mutation in exon 20. Both patient showed *KRAS* mutation -12D and were associated with better prognosis.

A clinicopathological comparison of *PIK3CA* patients and wild-type (wt) *PIK3CA* patients is presented in Table 1. The only statistically significant factor associated with *PIK3CA* mutations was MSI status. We also performed an analysis of *PIK3CA* mutation on the MSI positive subgroup. The clinicopathological analysis is presented in Table 2. Here, the only statistically significant factor was tumor position.

Table 1. *PIK3CA* wild type vs mutations in all GC group

Clinico-pathological characteristic	*PIK3CA* wt		*PIK3CA* mut		p-value
Patient (n)	462		10		–
Age (years, median)	70		75		0.058
Sex (male, female)	280:182 (60.6:39.4)		3:7 (30:70)		0.051
pT					0.657
1	42	9.1%	0	0%	–
2	74	16%	1	10%	–
3	95	20.6%	3	30%	–
4	251	54.3%	6	60%	–
pN					0.471
0	130	28.1%	3	30%	–
1	75	16.2%	2	20%	–
2	100	21.6%	4	40%	–
3a	74	16%	0	0%	–
3b	83	18%	1	10%	–
Tumor site					0.256
Non cardia	386	83.5%	7	70%	–
Cardia	76	16.5%	3	30%	–
MSI status					<0.001
MSS	360	77.9%	1	10%	–
MSI-H	102	22.1%	9	90%	–
Lauren					0.078
Diffuse/mixed	153	33.1%	0	0%	–
Intestinal	305	66%	10	100%	–
Unclassified	4	0.9%	0	0%	–
UICC-R					0.345
R0	339	73.4%	6	60%	–
R+	123	26.6%	4	40%	–
Stage					0.389
I	74	16%	1	10%	–
II	108	23.4%	3	30%	–
III	194	42%	6	60%	–
IV	86	18.6%	0	0%	–
M					0.131
M0	376	81.4%	10	100%	–
M1	86	18.6%	0	0%	–
WHO histological type*					0.132
Papillary	15	3.2%	0	0%	–
Poorly differentiated	146	31.6%	5	50%	–
Signet ring cell & mucinous	138	29.9%	0	0%	–
Tubular (well/mod. diff.)	153	33.1%	4	40%	–
Adjuvant					0.406
No	216	46.8%	6	60%	–
Yes	246	53.2%	4	40%	–

* 11 cases with unclassified WHO histotype are excluded ; MSS – microsatellite stable; MSI-H – microsatellite instable; M – metastases; pT – pathological tumor status; pN – pathological lymph node status.

Table 2. MSI status patients and *PIK3CA* mutations

Clinico-pathological characteristic	*PIK3CA* wt		*PIK3CA* mut		p-value
Patient (n)	102		9		–
Age (years, median)	75		76		0.559
Sex (male, female)	44:58 (43.1: 56.9)		2:7 (22.2:77.8)		0.222
pT					0.744
1	5	4.9%	0	0%	–
2	20	19.6%	1	11.1%	–
3	36	35.3%	3	33.3%	–
4	41	40.2%	5	55.6%	–
pN					0.309
0	44	43.1%	3	33.3%	–
1	18	17.6%	2	22.2%	–
2	19	18.6%	4	44.4%	–
3a	10	9.8%	0	0%	–
3b	11	10.8%	0	0%	–
Tumor site					0.008
Non-cardia	99	97.1%	7	77.8%	–
Cardia	3	2.9%	2	22.2%	–
Lauren					0.069
Diffuse/mixed	28	27.5%	0	0%	–
Intestinal	74	72.5%	9	100%	–
UICC-R					0.289
R0	83	81.4%	6	66.7%	–
R+	19	18.6%	3	33.3%	–
Stage					0.584
I	17	16.7%	1	11.1%	–
II	40	39.2%	3	33.3%	–
III	36	35.3%	5	55.6%	–
IV	9	8.8%	0	0%	–
M					0.353
M0	93	91.2%	9	100%	–
M1	9	8.8%	0	0%	–
WHO histological type*					0.287
Papillary	1	1%	0	0%	–
Poorly differentiated	42	41.2%	4	44.4%	–
Signet ring cell & Mucinous	23	22.5%	0	0%	–
Tubular (well/mod diff.)	34	33.3%	4	44.4%	–
Adjuvant					0.756
No	73	71.6%	6	66.7%	–
Yes	29	28.4%	3	33.3%	–

* 3 cases with unclassified WHO histotype are excluded ;
MSS – microsatellite stable; MSI-H – microsatellite instability;
pT – pathological tumor status; pN – pathological lymph node status;
UICC-R – Union Internationale Contre le Cancer Resection margin.

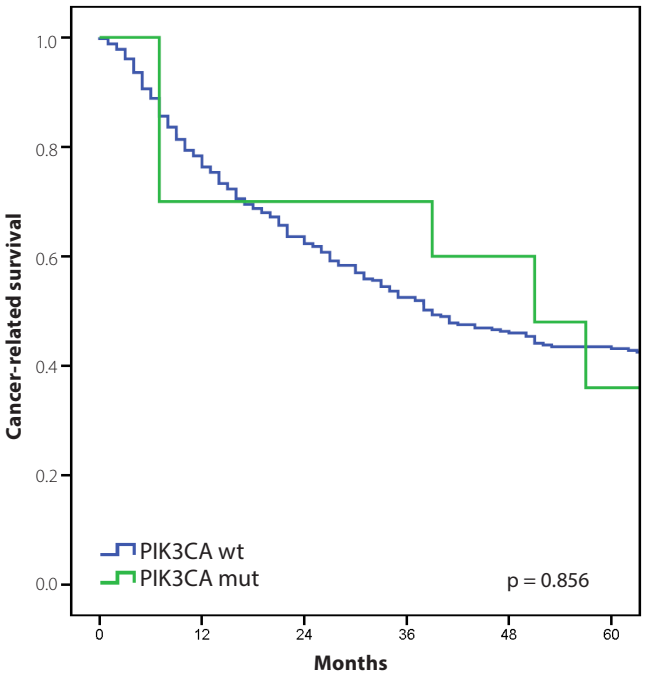

Fig. 1. Cancer-related survival of patients with or without mutation in *PIK3CA*

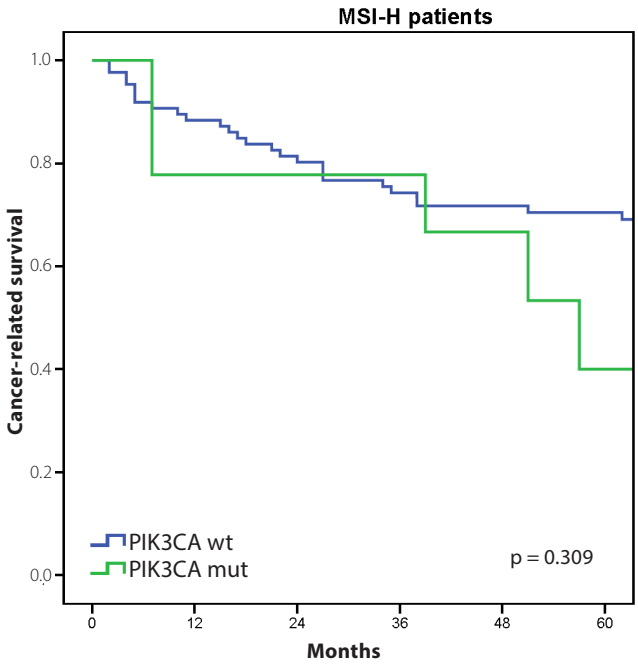

Fig. 2. Cancer-related survival of patients with MSI status with or without *PIK3CA* mutation

Interestingly all ten patients showed Lauren intestinal histotype.

We also analyzed cancer-related survivals. The first comparison was performed between all patients with or without *PIK3CA* mutation (Fig. 1). 5-year survival for *PIK3CA* wt patients was 43.2% and for *PIK3CA* mutation, 36% (p = 0.856). Secondly, we analyzed the group of MSI GC patients (Fig. 2). The 5-year survival of the MSI patients presenting with *PIK3CA* mutation was 40% and

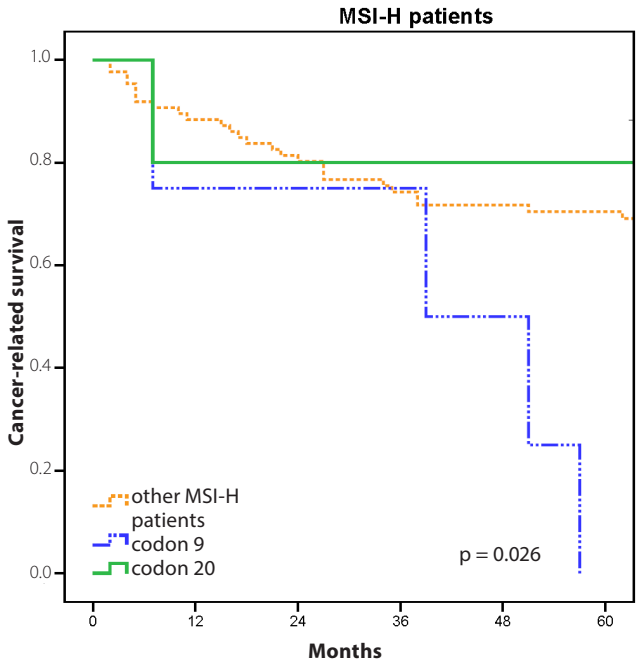

Fig. 3. Comparison of cancer-related survival of patients with different *PIK3CA* exon mutations together with a survival curve of MSI GC patients without *PIK3CA* mutation

Table 3. Cox proportional hazards regression analysis

Clinico-pathological characteristic	B	Exp (B)	95% CI	p-value
PIK3CA	–0.113	0.893	0.440–1.815	0.755
MSI status	–0.592	0.553	0.405–0.7554	<0.001
Age	0.032	1.032	1.018–1.047	<0.001
Gender	0.363	1.437	1.130–1.829	0.003
Lauren histotype	0.064	1.066	0.823–1.381	0.629
Tumor location	0.027	1.028	0.758–1.394	0.860
"T" parameter	0.602	1.826	1.322–2.522	<0.001
"N" parameter	0.675	1.965	1.354–2.850	<0.001
"M" parameter	0.060	1.062	0.770–1.463	0.715
Radicality of resection	1.098	2.999	2.264–3.972	<0.001
Adjuvant therapy	–0.033	0.967	0.683–1.370	0.851

PIK3CA status expressed as *PIK3CA* wt = 0 and *PIK3CA* mutation = 1; MS status expressed as MSS = 0 and MSI = 1; gender expressed as F = 0 an M = 1; Lauren histotype expressed as intestinal = 0 and non-intestinal = 1; tumor location expressed as non-cardias = 0 and cardias = 1; "T" parameter expressed as T1–T2 = 0 and T3–T4 = 1; "N" parameter expressed as N– = 0 and N+ = 1; "M" parameter expressed as negative = 0 and positive = 1; radicality of resection expressed as R0 = 0 and R1–R2 = 1; adjuvant therapy expressed as yes = 1 and no = 0;
MSI status – microsatellite instability; T parameter – pathological tumor status; N parameter – pathological lymph node status; M parameter – metastases status.

without the mutation, 70.4% (p = 0.309). We also checked the difference in survival of patients with the *PIK3CA* mutation in different exons (Fig. 3). For mutation in exon 9, the 5-year survival was 0% and for mutation in exon 20 – 80% (p = 0.031). For better visualization of these differences between the 2 mutation locations, we added a survival curve for MSI patients without *PIK3CA* mutation (p = 0.026) – Fig. 3.

The Cox proportional hazards regression analysis showed that age, sex, MSI status, "T" parameter, "N" parameter and type of "R" resection were the only prognostic factors statistically correlated with a worse overall survival (Table 3).

Discussion

In previous reports, the frequency of GC *PIK3CA* mutations varies from 4% to 13.2%.[21,22] In our paper, *PIK3CA* mutation occurred in 8.1% of the MSI group. In total, only a frequency of 2.1% was observed, lower in comparison with previous studies. The debate about *PIK3CA* mutation and its prognosis for different cancers is unresolved. Some authors suggest *PIK3CA* mutation is associated with a better prognosis in the case of breast cancer, while other authors showed worse prognosis in cancers like colorectal, endometrial, and lung cancers.[8,23–25] Warneke et al. presented interesting data for gastric cancer, showing worse survival in *PIK3CA* exon 20 mutation with intestinal histotype and better survival for the same mutation with diffuse histotype.[26] These results were statistically significant. In our study, it was impossible to analyze this factor because we did not observe diffuse/mixed histotype in our sample. *PIK3CA* mutation in exon 20 was found to be an independent prognostic factor of survival for intestinal pathology. Also, our results show that mutation in exon 20 presented improved patient survival.

In the paper by Fang et al., they found *PIK3CA* mutation in 57/432 of patients (13.2%).[22] They analyzed PIK3/AKT mutations together and found that in the intestinal histotype, patients presenting mutation in that gene showed tumors located mostly in the lower third of the stomach. In diffuse histotype, the location of the tumor was in the upper third of the stomach and patients showed a higher rate of hematogenous metastases. The authors did not find any difference in survival between patients presenting or not presenting *PIK3/AKT* mutation.

The authors also searched for a link between the *PIK3CA* mutation and Epstein-Barr virus (EBV) infection. The rate of EBV infection was higher only in the situation where the tumor was situated in the middle part of the stomach for GC patients with *PIK3CA* mutation.[22]

In a paper by Barbie et al., the authors also analyzed *PIK3CA* mutation in GC and its association with GC and MSI.[27] Only 8 of 39 MSI GC cases harbored the *H1047R* mutation. They found that this finding did not correlate with survival or any other clinical or pathological features linked with MSI GC. This is similar to our results on the exon 20 mutation in MSI patients, in which similar survival results were observed. A worse outcome was only observed in the case of exon 9 mutation, which was not observed in the above-mentioned study.[28]

In our study, we observed that *PIK3CA* patients were older and showed intestinal histotype more often but without statistical significance. Analyzing the subgroup of MSI GC patients, the only differences were tumor position (p = 0.008) and Lauren histotype (p = 0.069) – *PIK3CA* mutations were more commonly seen in the upper third of the stomach and also showed only intestinal histology. We did not observe any difference in survival between wild-type and mutated *PIK3CA* GC patients; however, in the MSI GC subgroup of patients, those with *PIK3CA* mutation had a worse 5-year survival rate (40%) than those without the mutation (70.4%).

PIK3CA mutations are also observed in head and neck squamous cell carcinoma (HNSCC) for 6–21% of patients.[4] Interestingly, this mutation was absent across German, Vietnamese, and Greek patients.[28,29] Likely, some ethnic, environmental, and/or other unknown factors are associated with this finding. A paper by Seiwert et al. pointed out that *PIK3CA* mutations are more commonly associated with human papillomavirus (HPV) positive HNSCC cancers.[30] This finding did not reach statistical significance but can be an example of a factor that may play an important role in developing this mutation. In our study, we observed one of the smallest incidences of *PIK3CA* mutation in GC patients – 2.1%.

In a CRC study by Day et al., it was found that *PIK3CA* exon 20 mutations were associated with proximal tumors and a sessile-serrated pathway (MSI-H/CIMP high/BRAF mutations), and *PIK3CA* exon 9 mutations were linked with the traditional serrated pathway of tumorigenesis (CIMP-low/KRAS mutations).[6] *PIK3CA* mutations were significantly associated with older age, proximal tumor site and mucinous histology, and *KRAS* mutation.[6] Comparison between wt *PIK3CA* and either exon 9 or 20 mutations showed some significant results. Mucinous histology was associated with exon 20, and for exon 9, factors like older age and *KRAS* mutations were associated. The direct comparison of exon 9 and 20 mutations did not reach statistical significance. In a study by Sukawa et al., MSI status was observed in 50% of *PIK3CA* GC patients.[21] Similarly, our results show a statistically significant link between *PIK3CA* mutation and MSI status. In fact, almost all of our GC patients presenting with a *PIK3CA* mutation were also MSI positive. *KRAS* mutation was observed only in the exon 20 mutation group (2 of 5 patients – 40%).

In breast cancer, *PIK3CA* mutations are seen in 40% of cases.[4] The presence of this mutation is associated with better prognosis in this cancer. Also, clinicopathological factors show higher rates of small tumor size, low grade, and positive estrogen receptor much more frequently in this group of patients.[24] Importantly, these patients also showed better survival.[24] In other cancers like colorectal, endometrial, or lung cancer, *PIK3CA* mutation is associated with worse prognosis.[23,25] We did not find any difference in survival between patients with and without *PIK3CA* mutations. The difference was observed when we analyzed subgroups according to the type of exon mutation.

Our study was limited by the small number of patients with *PIK3CA* mutations in our GC patient pool. We presented a link between different *PIK3CA* exon mutations and MSI GC that present completely different prognoses depending on the type of mutation. The MSI subtype of GC is a relatively new molecular subgroup and requires further analysis of different mutations that may have a positive or negative impact on patient outcome.

Our research leads to some important conclusions about *PIK3CA* mutations. Firstly, *PIK3CA* mutations in GC is rare. It is strongly associated with the MSI molecular subgroup, presenting a worse outcome than wt PIK3CA MSI GC patients. A completely different outcome is associated with mutation in exon 9 vs exon 20, with the latter being more favorable. The role of this mutation must be further studied with larger groups of patients.

References

1. Cristescu R, Lee J, Nebozhyn M, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med*. 2015;21(5):449–456.
2. Wadhwa R, Song S, Lee JS, Yao Y, Wei Q, Ajani JA. Gastric cancer-molecular and clinical dimensions. *Nat Rev Clin Oncol*. 2013;10: 643–655.
3. Willems L, Tamburini J, Chapuis N, Lacombe C, Mayeux P, Bouscary D. PI3K and mTOR signaling pathways in cancer: New data on targeted therapies. *Curr Oncol Rep*. 2012;14:129–138.
4. Lai K, Killingsworth MC, Lee CS. Gene of the month: PIK3CA. *J Clin Pathol*. 2015;68:253–257.
5. Volinia S, Hiles I, Ormondroyd E, et al. Molecular cloning, cDNA sequence, and chromosomal localization of the human phosphatidylinositol 3-kinase p110 alpha (PIK3CA) gene. *Genomics*. 1994;24: 472–477.
6. Day FL, Jorissen RN, Lipton L, et al. PIK3CA and PTEN gene and exon mutation-specific clinicopathologic and molecular associations in colorectal cancer. *Clin Cancer Res*. 2013;19(12):3285–3296.
7. Whitehall VL, Rickman C, Bond CE, et al. Oncogenic PIK3CA mutations in colorectal cancers and polyps. *Int J Cancer*. 2012;131: 813–820.
8. Mao C, Yang ZY, Hu XF, Chen Q, Tang JL. PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in KRAS wild-type metastatic colorectal cancer: A systematic review and meta-analysis. *Ann Oncol*. 2012;23:1518–1525.
9. Tapia O, Riquelme I, Leal P, et al. The PI3K/AKT/mTOR pathway is activated in gastric cancer with potential prognostic and predictive significance. *Virchows Arch*. 2014;465:25–33.
10. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513(7517): 202–209.
11. Isa N. Evidence based radiation oncology with existing technology. *Rep Pract Oncol Radiother*. 2014;19(6):259–266.
12. Polom W, Markuszewski M, Rho YS, Matuszewski M. Usage of invisible near infrared light (NIR) fluorescence with indocyanine green (ICG) and methylene blue (MB) in urological oncology. Part 1. *Cent European J Urol*. 2014;67(2):142–148.
13. Subhash VV, Yeo MS, Tan WL, Yong WP. Strategies and advancements in harnessing the immune system for gastric cancer immunotherapy. *J Immunol Res*. 2015;308574. doi: 10.1155/2015/308574
14. Alocci D, Mariethoz J, Horlacher O, Bolleman JT, Campbell MP, Lisacek F. Property Graph vs RDF triple store: A comparison on glycan substructure search. *PLoS One*. 2015;10(12):e0144578. doi: 10.1371/journal.pone.0144578
15. Mereiter S, Magalhães A, Adamczyk B, et al. Glycomic analysis of gastric carcinoma cells discloses glycans as modulators of RON receptor tyrosine kinase activation in cancer. *Biochim Biophys Acta*. 2016; 1860(8):1795–1808.

16. Beghelli S, de Manzoni G, Barbi S, et al. Microsatellite instability in gastric cancer is associated with better prognosis in only stage II cancers. *Surgery*. 2006;139:347–356.
17. Corso G, Pedrazzani C, Marrelli D, Pascale V, Pinto E, Roviello F. Correlation of microsatellite instability at multiple loci with long-term survival in advanced gastric carcinoma. *Arch Surg*. 2009;144: 722–727.
18. Marrelli D, Polom K, Pascale V, et al. Strong prognostic value of microsatellite instability in intestinal type non-cardia gastric cancer. *Ann Surg Oncol*. 2016;23(3):943–950.
19. Velho S, Oliveira C, Ferreira A, et al. The prevalence of *PIK3CA* mutations in gastric and colon cancer. *Eur J Cancer*. 2005;41:1649–1654.
20. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*. 1998;58(22):5248–5257.
21. Sukawa Y, Yamamoto H, Nosho K, et al. Alterations in the human epidermal growth factor receptor 2-phosphatidylinositol 3-kinase-v-Akt pathway in gastric cancer. *World J Gastroenterol*. 2012;18(45): 6577–6586. doi: 10.3748/wjg.v18.i45.6577
22. Fang WL, Huang KH, Lan YT, et al. Mutations in *PI3K/AKT* pathway genes and amplifications of *PIK3CA* are associated with patterns of recurrence in gastric cancers. *Oncotarget*. 2016;7(5):6201–6220. doi: 10.18632/oncotarget.6641
23. Catasus L, Gallardo A, Cuatrecasas M, Jaime P. *PIK3CA* mutations in the kinase domain (exon 20) of uterine endometrial adenocarcinomas are associated with adverse prognostic parameters. *Mod Pathol*. 2008;21:131–139.
24. Kalinsky K, Jacks LM, Heguy A, Patil S, Prat J. *PIK3CA* mutation associates with improved outcome in breast cancer. *Clin Cancer Res*. 2009; 15:5049–5059.
25. Janku F, Garrido-Laguna I, Petruzelka LB, Stewart DJ, Kurzrock R. Novel therapeutic targets in non-small cell lung cancer. *J Thorac Oncol*. 2011;6:1601–1612.
26. Warneke VS, Behrens HM, Haag J, et al. Prognostic and putative predictive biomarkers of gastric cancer for personalized medicine. *Diagn Mol Pathol*. 2013;22(3):127–137. doi: 10.1097/PDM0b013e318284188e
27. Barbi S, Cataldo I, De Manzoni G, et al. The analysis of *PIK3CA* mutations in gastric carcinoma and metaanalysis of literature suggest that exon-selectivity is a signature of cancer type. *J Exp Clin Cancer Res*. 2010;16:29–32.
28. Kostakis GC, Papadogeorgakis N, Koumaki V, Kamakari S, Koumaki D, Alexandridis C. Absence of hotspot mutations in exons 9 and 20 of the *PIK3CA* gene in human oral squamous cell carcinoma in the Greek population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109:e53–58. doi: 10.1016/j.tripleo.2010.01.015
29. Fenic I, Steger K, Gruber C, Arens C, Woenckhaus J. Analysis of *PIK-3CA* and Akt/protein kinase B in head and neck squamous cell carcinoma. *Oncol Rep*. 2007;18:253–259.
30. Seiwert, TY, Zuo Z, Keck MK, et al. Integrative and comparative genomic analysis of HPV-positive and HPV-negative head and neck squamous cell carcinomas. *Clin Cancer Res*. 2015;21(3):632–641. doi: 10.1158/1078-0432.CCR-13-3310