

Clinical Evidence for Three Distinct Gastric Cancer Subtypes: Time for a New Approach

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Abstract

Background: Recently, a new classification for gastric cancer (GC) has been proposed, based on Lauren's histology and on anatomic tumour location, identifying three subtypes of disease: type 1 (proximal non diffuse GC), type 2 (diffuse GC) and type 3 (distal non diffuse GC). Aim of our analysis was to compare clinical outcome according to different GC subtypes (1,2,3) in metastatic GC patients receiving first-line chemotherapy.

Patients and Methods: Advanced GC pts treated with a first-line combination chemotherapy were included in our analysis. Pts were divided in three subgroups (type 1, type 2 and type 3) as previously defined.

Results: A total of 248 advanced GC pts were included: 45.2% belonged to type 2, 43.6% to type 3 and 11.2% to type 1. Patients received a fluoropyrimidine-based chemotherapy doublet or three drugs regimens including a platinum derivate and a fluoropyrimidine with the addition of an anthracycline, a taxane or mytomicin C. RR was higher in type 1 pts (RR=46.1%) and type 3 (34.3%) compared to type 2 (20.4%), ($p=0.015$). Type 2 presented a shorter PFS, median PFS=4.2 months, compared to type 1, mPFS=7.2 months, and type 3, mPFS=5.9 months ($p=0.011$) and also a shorter OS ($p=0.022$).

Conclusions: Our analysis suggests that GC subtypes may be important predictors of benefit from chemotherapy in advanced GC patients. Future clinical trials should take in account these differences for a better stratification of patients.

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Background

Despite its incidence in Europe and North America has declined over the last three decades, gastric cancer (GC) is still the second cause of cancer-related deaths worldwide [1], representing a challenging problem for oncologists. Although different histological subtypes of GC have been identified, in the daily clinical practice and for the purpose of medical management, GC is usually considered as a single disease.

The World Health Organization and Lauren's classification system have described two main histological types of gastric cancer, intestinal and diffuse subtype, representing two entirely different epidemiological and pathological entities. The intestinal-type, characterized by cohesive neoplastic cells organized in gland-like tubular structures, is more common in men and older people; it usually arises following chronic infection by *H. Pylori* with consequent chronic inflammation and atrophic gastritis [2,3]. On the other hand, diffuse GC, histologically characterized by infiltration and thickening of the stomach wall, occurs more commonly in women and young patients; it is usually independent from inflammation processes and can be hereditary, as a result of germline mutation of E-cadherin [4]. The gastro-esophageal

junction (GEJ) cancer, arising from proximal stomach, is currently considered as a third distinct entity. Despite the overall decrease in GC incidence, the incidence of GEJ adenocarcinoma in Western countries has increased over the last decades. Gastroesophageal reflux disease and obesity are considered the major risk factors for GEJ cancer and Barrett esophagus represents a precancerous lesion for this type of tumour (Table 1).

This classification is also supported by clinical differences between the GC subtypes. In particular, it is well known that intestinal and diffuse GC have different metastatic pattern, with diffuse type more likely to spread to the peritoneum compared to intestinal GC. [5]. It has also been demonstrated that patients with resected diffuse GC have a worse clinical outcome, when compared stage-by-stage, to intestinal GC patients [6].

Recently, these epidemiological, pathological and clinical data have been incorporated to define a new classification of GC that identifies three tumour subtypes: type 1, proximal non-diffuse GC, with the bulk of the tumour (>80%) located in the gastric cardia and characterized by a non-diffuse pattern of infiltration; type 2, diffuse GC, located anywhere in the stomach with an entirely diffuse pattern of infiltration; type 3, distal non-diffuse GC, with the bulk of the tumour located in the distal or mid stomach and a

Table 1. Gastric cancer subtypes features.

Gastric Cancer Subtype	Prevalent Risk Factor	
Proximal, Non-Diffuse GC (type 1)	<i>Environmental</i>	Tobacco use, Alcohol
	<i>Clinical</i>	Obesity, High BMI, GERD
	<i>Genetic</i>	Non specifically identified
Diffuse GC (type 2)	<i>Environmental</i>	Non specifically identified
	<i>Clinical</i>	unknown
	<i>Genetic</i>	CDH1 mutation, Family history (non CDH1 mutant)
Distal, Non-Diffuse GC (type 3)	<i>Environmental</i>	High dietary salt, Tobacco, Age (peak 50–70 yrs), Low Fruit/vegetables intake
	<i>Clinical</i>	<i>H.Pylori</i> infection, Use of NSAIDs
	<i>Genetic</i>	Immune regulatory SNPs

Modified from Shah et al [8].

BMI: Body Mass Index, GERD: Gastroesophageal Reflux Disease, CDH1: Cadherin-1, NSAIDs: Non-Steroidal Anti-Inflammatory Drugs, SNPs: Single Nucleotide Polymorphism.
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dominant pattern of intestinal type carcinoma [7]. Furthermore, Shah et al have demonstrated that these GC subtypes, classified on the basis of histology and anatomic location, have also distinct gene expression profiles, supporting the hypothesis that GC subtypes may be distinguished molecularly [8].

One of the clinical implications of this heterogeneity in GC biology is the possible different sensitivity to chemotherapy treatment among the different GC subtypes. Currently, the choice of medical treatment for advanced GC does not take into account the clinical and pathological heterogeneity of this disease. Nevertheless, differences in response to treatment between different subtypes have been reported by a subset analysis of the FLAGS trial, which showed better overall for patients with diffuse GC when treated with cisplatin/S-1 compared to cisplatin/5-FU [9].

Aim of our analysis was to compare the clinical outcome, in terms of response rate, RR, progression-free survival, PFS, and overall survival, OS according to different GC subtypes (1,2,3) in advanced gastric cancer patients receiving first-line platinum-based chemotherapy.

Patients and Methods

Patients Selection

The study population was selected from a central database including patients with gastric cancer treated and followed at our institution. Clinical data were retrieved from medical charts. Patients with histologically confirmed, inoperable locally advanced, recurrent or metastatic gastric or gastroesophageal junction adenocarcinoma who have received a first-line combination chemotherapy with a two or three-drugs regimen were included in our analysis. Patients were eligible if they had measurable or non measurable disease; Eastern Cooperative Oncology Group performance status 0–2; age ≥ 18 ; no central nervous system metastasis. Patients with HER-2 positive gastric cancer, including patients treated with trastuzumab, were excluded from our analysis.

The patients were divided in three subtypes as previously described:

- type 1, proximal non-diffuse GC, with the bulk of the tumour (>80%) located in the gastric cardia, with possible extension to the distal part of the esophagus, and characterized by a non-diffuse pattern of infiltration;

- type 2, diffuse GC, located anywhere in the stomach with an entirely diffuse pattern of infiltration with no gland-forming, intestinal type of carcinoma;
- type 3, distal non-diffuse GC, with the bulk of the tumour located in the distal or mid part of the stomach and a dominant pattern of intestinal type carcinoma, with or without components of poorly differentiated carcinoma

Gastric cancer subtypes were determined retrospectively reviewing the pathological report for each patient.

Ethics Statement

This study was approved by Ethical committee AOU Ospedali Riuniti – Umberto I of our institution. All patients provided informed written consent.

Treatment and Response evaluations

Chemotherapy regimens administered to patients included: fluoropyrimidines and platinum based doublets, as cisplatin 5-fluorouracil (5-FU), cisplatin capecitabine, oxaliplatin FU (FOLFOX) or capecitabine oxaliplatin; 5-FU irinotecan (FOLFIRI) or 5-FU mytomicin C combinations; three drugs regimens including anthracyclines (epirubicin, fluoropyrimidines and a platinum derivate combinations as ECF, EOX, PELF), taxanes (docetaxel, fluoropyrimidines and a platinum derivate combinations as TCF or TOX) and mytomicin C (mytomicin C, fluoropyrimidines and a platinum derivate combinations). Physical examination, complete blood counts and biochemical tests were carried out before each cycle of therapy. A chest and abdomen CT scan was performed every three months and when disease progression was clinically suspected by the treating physician to document the extent of disease and to evaluate the response to treatment. The response was assessed using the Response Evaluation Criteria in Solid Tumours (RECIST) 1.0. After the end of treatment, patients were followed every three months with laboratory and imaging studies according to our internal guidelines. Disease status at last follow-up and cause of death were determined by the medical record and death certificates.

Statistical analysis

Patient, tumour, and treatment variables were compared between the three subgroups using the chi-square for categorical variables. For statistical analysis, overall survival (OS) and

progression-free survival (PFS) were defined, respectively, as the interval between the first day of first-line chemotherapy until the time of the first occurrence of progression, death from any cause or to the date of last follow-up visit and as the interval between the first day of first-line chemotherapy to the date of death or to the date of the last follow-up visit. Kaplan–Meier method was used to estimate PFS and OS curves in the three subgroups; PFS and OS were compared using the log-rank test and we used Cox-regression models for survival multivariate analysis. Tested variables included gender, ECOG PS (0–1 vs 2), stage (locally advanced vs metastatic disease), first-line chemotherapy (three drugs vs two drugs regimen), previous use of adjuvant or neoadjuvant treatment, peritoneal carcinosis, number of metastatic sites (1 vs ≥ 2), use of second line chemotherapy and disease subtype (1, 2 or 3) as previous defined. We compared the overall response rates (ORR) between the two groups, including complete response and partial response, using the chi-square test.

Results

Characteristics of the patients

A total of 248 advanced gastric cancer patients treated with chemotherapy between January 2003 and December 2011 were included in our analysis. Patients characteristics are summarized in Table 2. The majority of patients were males (65.3%); consistently with epidemiological data the proportion of female patients was found to be higher in group 2. The 11.3% of patients (28 patients) presented with proximal non-diffuse GC and were classified as type 1; 112 patients (45.2%) had a diagnose of diffuse GC and were classified as type 2, while type 3 GC, distal-non diffuse GC, included 108 patients (43.5%).

The 3 groups of patients resulted comparable for most of baseline characteristics of clinical relevance (age, ECOG PS, stage, number of metastatic sites). Most of the patients (91.9%) had metastatic disease while the remaining 8.1% presented with locally

advanced, unresectable disease. More than a half of patients (58.1%) had undergone previous surgery for their disease and 20.9% of the patients also received neoadjuvant or adjuvant chemotherapy. As expected, type 2 GC patients, presenting diffuse histology, had a higher incidence of peritoneal carcinosis compared to other groups (50% vs 29.6% in type 3 and 28.6% in type 1).

Treatment

One-hundred and forty-seven patients (59.3%) received a two drugs fluoropyrimidine-based chemotherapy regimen as first-line treatment. Most of the patients received a combination including a platinum derivate and a fluoropyrimidine (100 patients); the remaining 47 patients received a fluoropyrimidine and irinotecan (22 patients) or mytomicin C (25 patients).

One-hundred and one patients (40.7%) received a three drugs regimen as first-line combination chemotherapy, including a platinum derivate, a fluoropyrimidine and a third drug; in particular, 35 patients received an anthracyclines based combination, 27 patients received a taxanes based regimen while 39 patients received a triplet containing mytomicin. No significant difference was found between the three groups of patients in the use of three or two drugs regimen ($p = 0.18$). First-line regimens administered in the two groups of patients are summarized in Table 3. A total of 137 patients (55.2%) received a second line chemotherapy, with no significant differences between the three GC subtypes. FOLFIRI (5-fluorouracil, leucovorin and irinotecan) was the most commonly used regimen in this setting. Other second line treatments included taxanes (paclitaxel or docetaxel) and FOLFOX (5-fluorouracil, leucovorin and oxaliplatin).

Efficacy

In 223 patients with measurable disease, the overall response rate to first-line chemotherapy in patients was 29.6%. We observed significant differences in response rate according to

Table 2. Patients' Characteristics.

		Type 1 (28 pts)	Type 2 (112 pts)	Type 3 (108 pts)	
Age	Median (Range),ys	63 (51–76)	61 (29–85)	66 (39–81)	
Gender	M	24	60	78	$p = 0.0008$
	F	4	52	30	
ECOG PS	0–1	24	78	84	$p = 0.14$
	2	4	34	24	
Surgery	Yes	14	60	70	$p = 0.16$
	No	14	52	38	
Prior Neoadjuvant/Adjuvant Chemotherapy	Yes	2	28	22	$p = 0.11$
	No	26	84	86	
Stage	Locally advanced	2	10	4	$p = 0.28$
	Metastatic	26	102	104	
Metastatic Sites	1	12	54	62	$p = 0.55$
	≥ 2	14	48	42	
Peritoneal Carcinosis	Yes	8	56	32	$p = 0.004$
	No	20	56	76	
Second Line Chemotherapy	Yes	18	60	59	$p = 0.59$
	No	10	52	49	

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Table 3. First-line chemotherapy regimens administered in the three groups of patients.

	Type 1	Type 2	Type 3
	n. pts (%)	n. pts (%)	n. pts (%)
Two drugs regimens	16 (57.1%)	60 (53.6%)	71 (65.7%)
Fluoropyrimidine platinum derivate	12	40	48
Fluoropyrimidine irinotecan	4	8	10
Fluoropyrimidine mytomicin C	0	12	13
Three drugs regimens	12 (42.9%)	52 (46.4%)	37 (34.3%)
Anthracyclines-containing chemotherapy	4	18	13
Taxanes-containing chemotherapy	3	14	10
Mytomicin-containing chemotherapy	5	20	14

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disease subtype: the higher response rate was reported in subtype 1 (12/26 patients, 46.1%), while response rate was 34.3% in subtype 3 patients and 20.4% in patients with type 2 gastric cancer ($p=0.015$), as described in Table 4. Subtype 2 gastric cancer patients presented a shorter PFS compared to other subgroups with a median PFS of 4.2 months compared to a median PFS of 7.2 months for type 1 patients and 5.9 months for type 3 ($p=0.011$) (Figure 1). These differences translated in statistically significant differences in OS. In particular, median OS was 9.8 months in subtype 2 patients compared to a median OS of 11.5 months in subtype 1 and 11.0 months in subtype 3 patients ($p=0.022$) (Figure 2). At survival multivariate analysis, when we considered together the two disease subtypes with better outcome (type 1 and type 3), type 2 gastric cancer subtype was found to be an independent predictor for OS (HR = 1.41; 95% CI 1.02–1.94, $p=0.038$) together with previous surgery (HR = 0.55; 95% CI 0.39–0.79, $p=0.0012$), use of adjuvant or neoadjuvant treatment

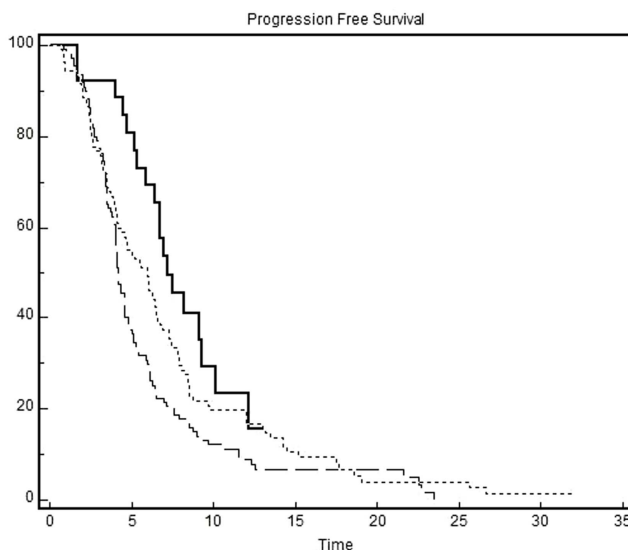


Figure 1. Progression-free survival according to subtypes. — type 1 proximal non diffuse GC (median PFS = 7.2 months). - - - type 2 diffuse GC (median PFS = 4.2 months). ····· type 3 distal non diffuse GC (median PFS = 5.9 months). $P=0.011$. doi:10.1371/journal.pone.0078544.g001

(HR = 0.57; 95% CI 0.35–0.90, $p=0.018$) and use of second line chemotherapy (HR = 0.53; 95% CI 0.39–0.73, $p=0.0001$).

Discussion

Advanced gastric cancer is an aggressive disease with high mortality rate. Combinations chemotherapy is the treatment of choice for advanced GC but despite recent progress in cancer treatment, patients' prognosis remains dismal. Currently, even if GC heterogeneity is well recognized, medical management of gastric cancer is not influenced by epidemiological, histological or anatomical considerations. Our analysis has shown how clinical outcome of advanced GC patients treated with chemotherapy is different in terms of RR, PFS and OS according to histology and tumour location, with proximal non-diffuse GC presenting the more favourable outcome.

Clinical implications of GC biological heterogeneity are increasingly being identified in recent trials. In a subset analysis of a phase II trial evaluating bevacizumab with a modified DCF regimen (docetaxel, cisplatin, 5-fluorouracil) in advanced GC patients [10] diffuse GC was shown to have significantly worse PFS and OS compared to other subtypes.

Diffuse tumours also presented the worse response rate with 38% compared to 56% of distal/body diffuse GC and 85% of proximal non-diffuse GC. Interestingly, in this trial gastroesophageal tumours, usually considered more aggressive and presenting a worse prognosis compared to tumours arising from the rest of the stomach [11,12] had a greater benefit from the treatment. Authors suggested that bevacizumab may be more active in this subset, proximal non-diffuse GC, and may improve the clinical outcome of patients overcoming the adverse prognosis characteristics. Along with these data, our analysis has shown an adverse prognosis for type 2 advanced GC patients treated with combination chemotherapy. Nevertheless, type 1 (proximal non-diffuse) GC patients in our study presented a lower response rate, about 46%, compared to that observed in the phase II trial by Shah et al. A possible explanation is that a more than a half of patients (59.3%) in our study did not receive a three-drugs chemotherapy regimen but a two drug combination and none of the patients received bevacizumab. Suggestions of variable efficacy of treatment on the basis of disease subtype in GC have been observed also in large phase III trial, such as the already cited FLAGS or the ToGA trial. The ToGA trial was an international phase III trial that randomized HER2 positive advanced GC patients to cisplatin and capecitabine/fluorouracil plus trastuzumab or to chemotherapy alone [13]. HER2 expression is more common in intestinal type tumours than in other subtypes [14] and indeed diffuse GC represented only 9% of all patients enrolled in the trial. A subset analysis showed that the addition of trastuzumab to chemotherapy in this subgroup of patients had no effect on survival with a HR for OS of 1.07 (0.56–2.05) versus a HR of 0.69 (0.54–0.88) for intestinal type GC patients.

Evidences of different response to treatment between GC subtypes have been reported not only in patients with advanced disease but also in the adjuvant setting. In particular, in a recent update of INT-0116 study, evaluating post-operative chemoradiotherapy in patients with resected GC, it has been shown that the benefit of adjuvant treatment is minimal in patients with diffuse histology while is significant in all the other subsets [15]. Similar findings have been observed also in the ITACA-S trial, a multicenter phase III trial comparing 5-fluorouracil and leucovorin versus a sequential regimen including irinotecan and 5-fluorouracil followed by cisplatin and docetaxel in the adjuvant treatment of resected GC patients. In a subgroups analysis of the

Table 4. Response Rate to first-line chemotherapy according to tumour subtype (in 223 patients with measurable disease).

Response Rate	Type 1	Type 2	Type 3
Overall RR	12/26 (46.1%)	20/98 (20.4%)	34/99 (34.3%)
Partial Response	12/26 (46.1%)	20/98 (20.4%)	30/99 (30.3%)
Complete Response	0/26 (0%)	0/98 (0%)	4/99 (4.0%)
Stable Disease	9/26 (35.7%)	34/98 (34.7%)	30/99 (30.3%)
Progressive Disease	5/26 (18.2%)	44/98 (44.9%)	35/99 (35.4%)

RR: Response Rate.
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trial, which could not demonstrate any benefit for the intensive treatment versus the fluorouracil monotherapy, the authors compared the outcome of patients according to disease subtype, dividing the patients in type 1, type 2 and type 3 as defined by Shah et al. The subtype 2, diffuse GC, presented a worse outcome in terms of OS compared to type 3 (HR = 1.35; 95% CI 1.06–1.72, $p = 0.016$) while no significant difference in terms of prognosis was found between subtype 1 and 3. None of the three subtypes showed a benefit for the experimental arm versus the 5-fluorouracil arm [16].

Although we think that our results are interesting and relevant, we acknowledge that a few points should be more accurately discussed. Firstly, our analysis excluded HER-2 positive GC patients. Even if we believe that also HER-2 positive GC requires a classification, we decided to exclude these patients from our study considering the different biology of this disease, the potential prognostic role of HER-2 expression and the possible confounding factor represented by trastuzumab treatment. The use of different chemotherapy regimens in the study population may affect the results, however we found no difference in the use of two or three drugs regimens between the three subtypes of patients. Moreover, the number of patients in some subgroups, in particular subtype 1 patients, is low and the difference observed in OS, although statistically significant, is actually small and of questionable clinical value. Nevertheless, in our study this difference in OS is associated with a relevant difference in response rate and PFS between the three subgroups of patients. Our analysis suggests that different GC subtypes may present different sensitivity to chemotherapy treatment. Future clinical trials, evaluating chemotherapy treatment in advanced GC patients, should take in account these differences for a better stratification of patients. Indeed, one of the possible explanation for the negative results of recent phase III trials evaluating new cytotoxic or target therapies, such as FLAGS or AVAGAST [17] is that GC is a heterogeneous disease with different biology that may affect response to treatment. Actually, preliminary evidences have shown that the three distinct GC subtypes identified on histopathologic and anatomic criteria, present different gene expression profiles [7]. In the last few years, several studies have demonstrated that molecular

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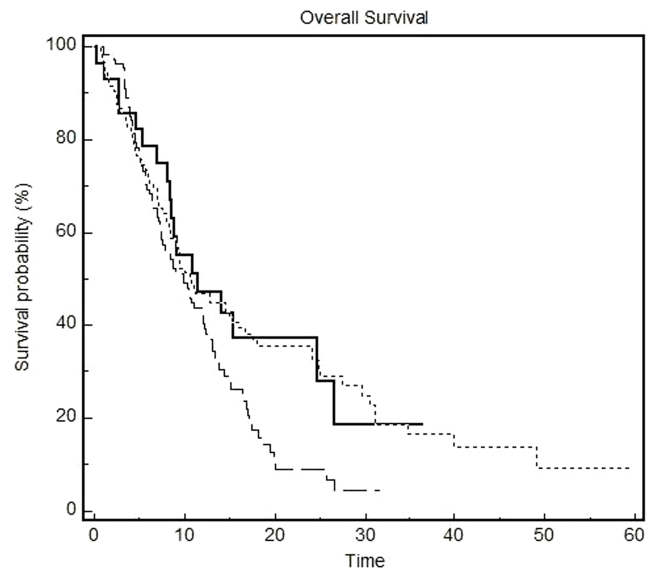


Figure 2. Overall survival according to subtypes. — type 1 proximal non diffuse GC (median OS = 11.5 months). - - - type 2 diffuse GC (median OS = 9.8 months). ····· type 3 distal non diffuse GC (median OS = 11.0 months). $P = 0.022$.
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markers may correlate to either response or toxicity to specific antineoplastic drugs in GC [18]. Differential expression of biological factors involved in chemotherapy activity, including targets of chemotherapeutic agents, such as thymidylate synthase (TS) for 5-FU, but also genes involved in drug metabolism, may explain the different response to treatment observed in our study. For example, a study by Kamoshida S et al assessed expression of TS, DPD (dihydropyrimidine dehydrogenase) and TP (thymidine phosphorylase) levels in different tumour types, including intestinal-type and diffuse-type gastric adenocarcinoma. The authors found high level of expression of TS, which may be associated with poor response to 5-FU based chemotherapy, in diffuse GC while TS was not overexpressed in intestinal type GC [19].

In conclusions, our study suggests that response to chemotherapy may be different in advanced GC patients, according to tumour histology and anatomic location. Genetic and translational studies are warranted to improve the understanding of molecular drivers and pathways of different GC subtype that may help to identify prognostic and predictive biomarkers as well as to identify specific targets for therapy.

Author Contributions

Conceived and designed the experiments: AB MS RG SC. Performed the experiments: IB AM AB RG LF MDP LC MP. Analyzed the data: AB MP MS RG MB LF AM. Contributed reagents/materials/analysis tools: IB AM MB MDP LC AB. Wrote the paper: AB MS SC RG LF MP.

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