



Molecular Characterization of Bladder Cancer in Smokers versus Nonsmokers



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Abstract #4528

Background

- Bladder cancer (BC) is one of the most common malignancies of the urinary tract and is the 4th most common cancer among men.
- It is estimated that by the end of 2015, the US will have approximately 74,000 new BC cases, accounting for 16,000 cancer-related deaths.
- Smoking is considered an important risk factor for BC. Recent data demonstrate an increase in BC incidence in nonsmokers as well.
- Molecular characterization of BC in nonsmokers has not been well studied.

Methods

- 676 consecutive BC profiled at a CLIA-certified laboratory from 2006 through 2014 were evaluated for differences in molecular characterization between smokers and nonsmokers.
- Smoking status (nonsmokers [NS]; current or reformed smokers [R/S]), patient characteristics, age, sex and survival data were collected on each subgroup.
- Formalin fixed paraffin-embedded (FFPE) samples were analyzed. Tumors were verified by a board-certified pathologist to confirm diagnosis.
- Protein expression was determined by IHC analysis, using commercially available detection kits and automated staining techniques. *In-situ* hybridization, fluorescent (FISH) or chromogenic (CISH), was used for evaluation of *HER-2/neu* and *EGFR*. *ALK* rearrangements, either inversion or translocation of the *ALK* gene at 2p23, were identified by separation of the fusion signals.
- Gene sequencing was performed by next-generation sequencing (NGS). Full details are available at www.carislifesciences.com.

Results

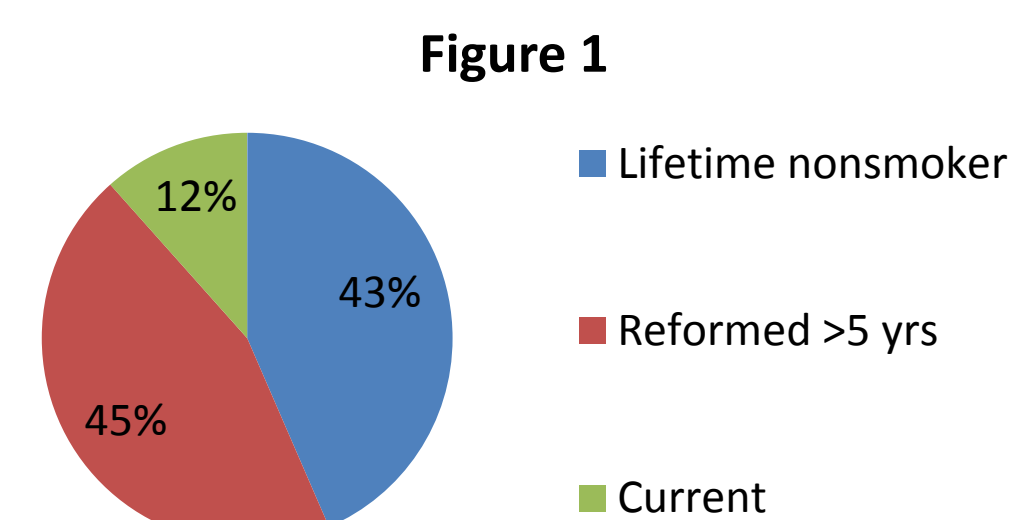
- Identified trends included differences in the PI3 kinase, Wnt and EGFR pathways.
 - Percentage of *PIK3CA* mutations was higher in NS (43%) than R/S (11%).
 - Wnt pathway aberrations (e.g. *CTNNB1* and *APC* mutations) occurred more frequently in R/S.
 - EGFR* amplification occurred in 22% NS but only 11% in R/S.
 - HER2* was amplified only in R/S (23% vs. 0%, $p=0.05$).
- Three of eight (37.5%) R/S had an *ALK* 2p23 rearrangement. For comparison, *ALK* is found in ~5% non-small cell lung cancers.
- TP53* did not differ between the populations.
- Survival data from 31 patients (14 NS, 17 R/S) showed overall average survival in the NS cohort was 175 days longer than in the R/S cohort.

Patient Demographics

Table 1. Categorization of cases and gender/age breakdown.

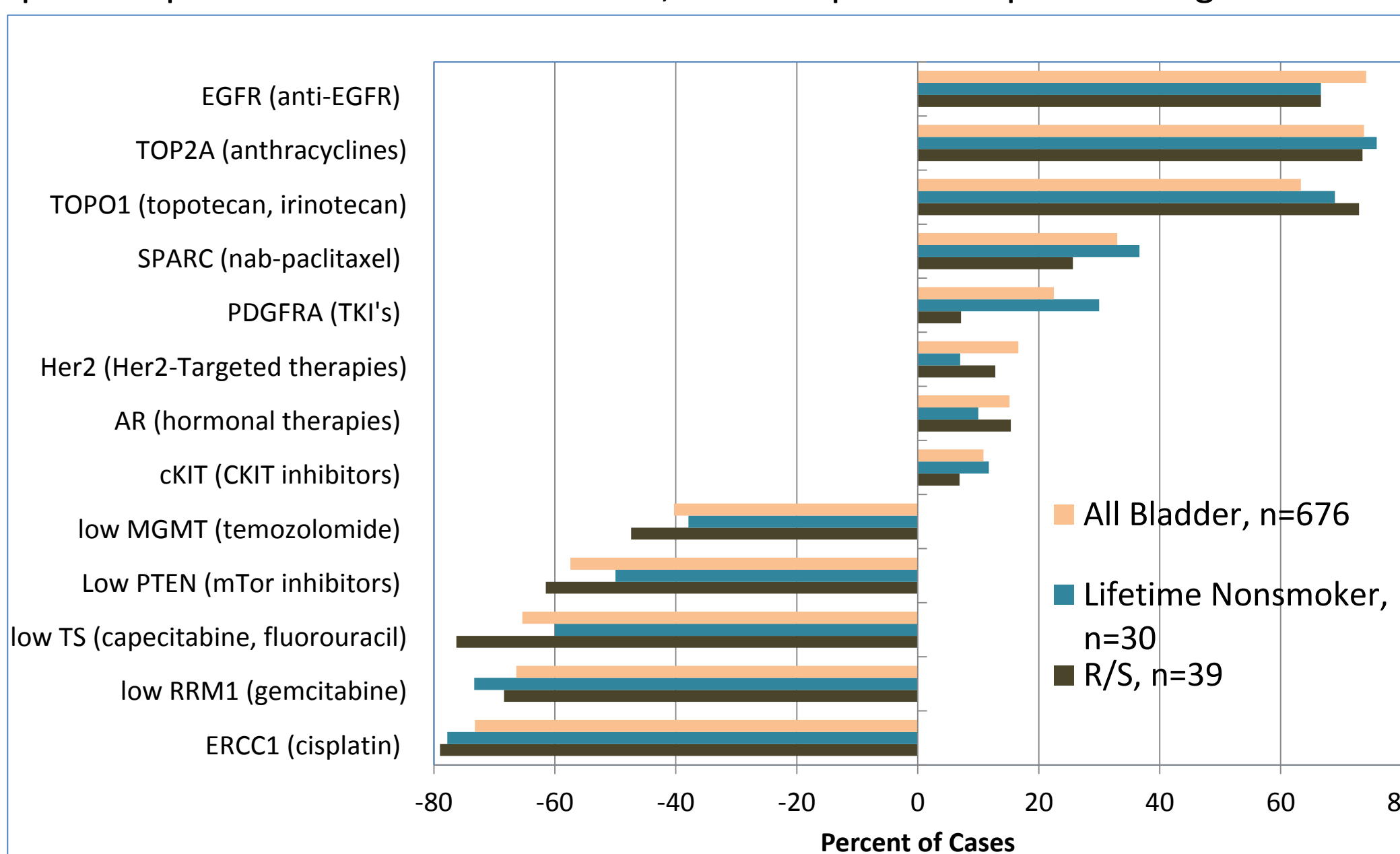
Category; Total Cases	Gender; Age
All bladder cases, n=676	M: 75%; F: 25% Median age: 66 (range 20-95)
Lifetime nonsmoker, n=30	M: 21%; F: 9% Median age: 66
Current smoker, n=8	M: 6%; F: 2% Median age: 65
Reformed smoker, > 5 yr, n=31	M: 22%; F: 9% Median age: 69
Grouped, total current or reformed smoker (R/S), n=39	

Figure 1. Distribution of 69 cases with known smoking status. Data were provided by participating physicians. Most were current or reformed smokers (55%).



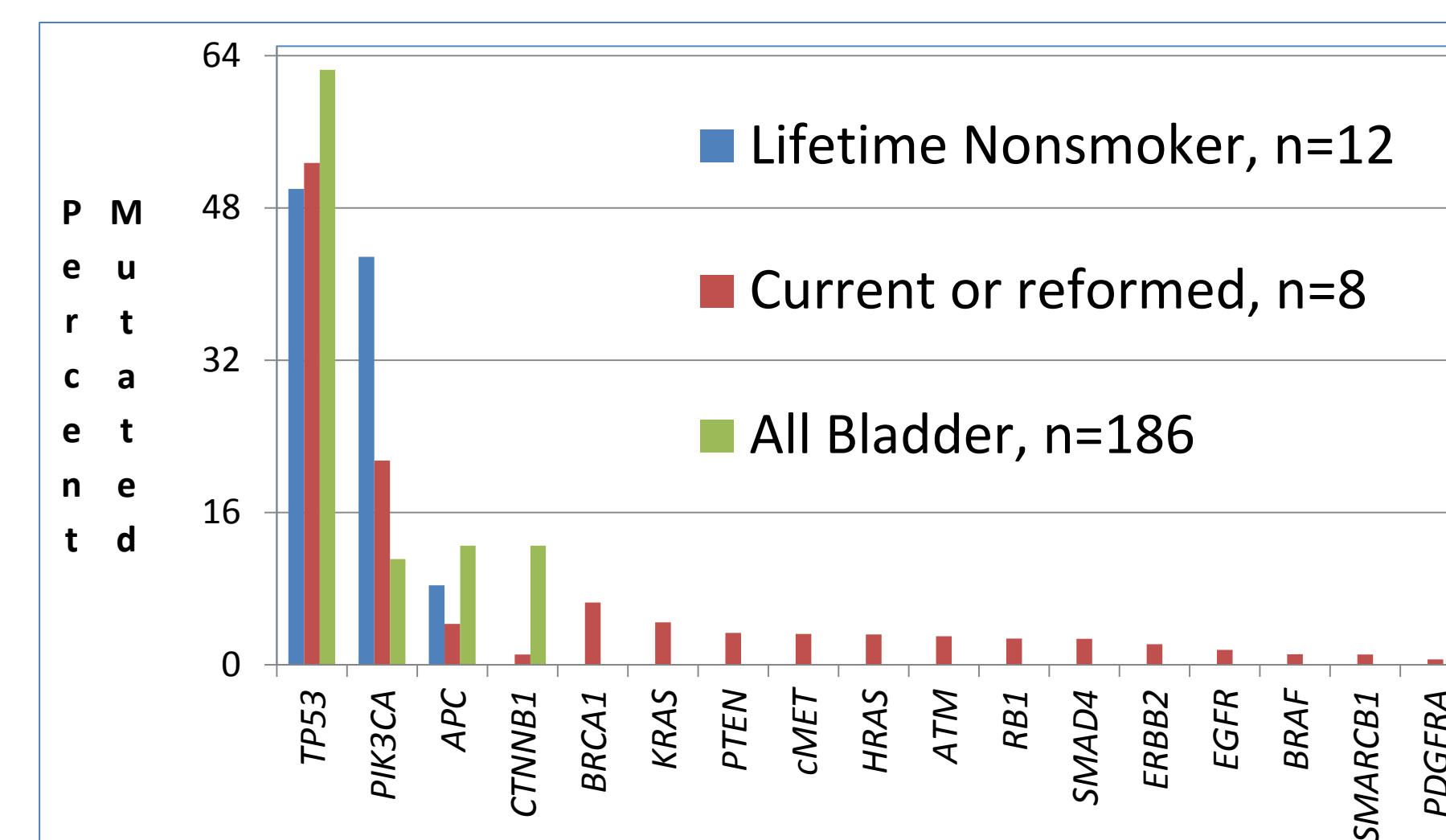
Results, Immunohistochemistry (IHC)

Figure 2. Levels of protein expression. Overexpression is reported as percent positive of total cases tested; loss is reported as percent negative.



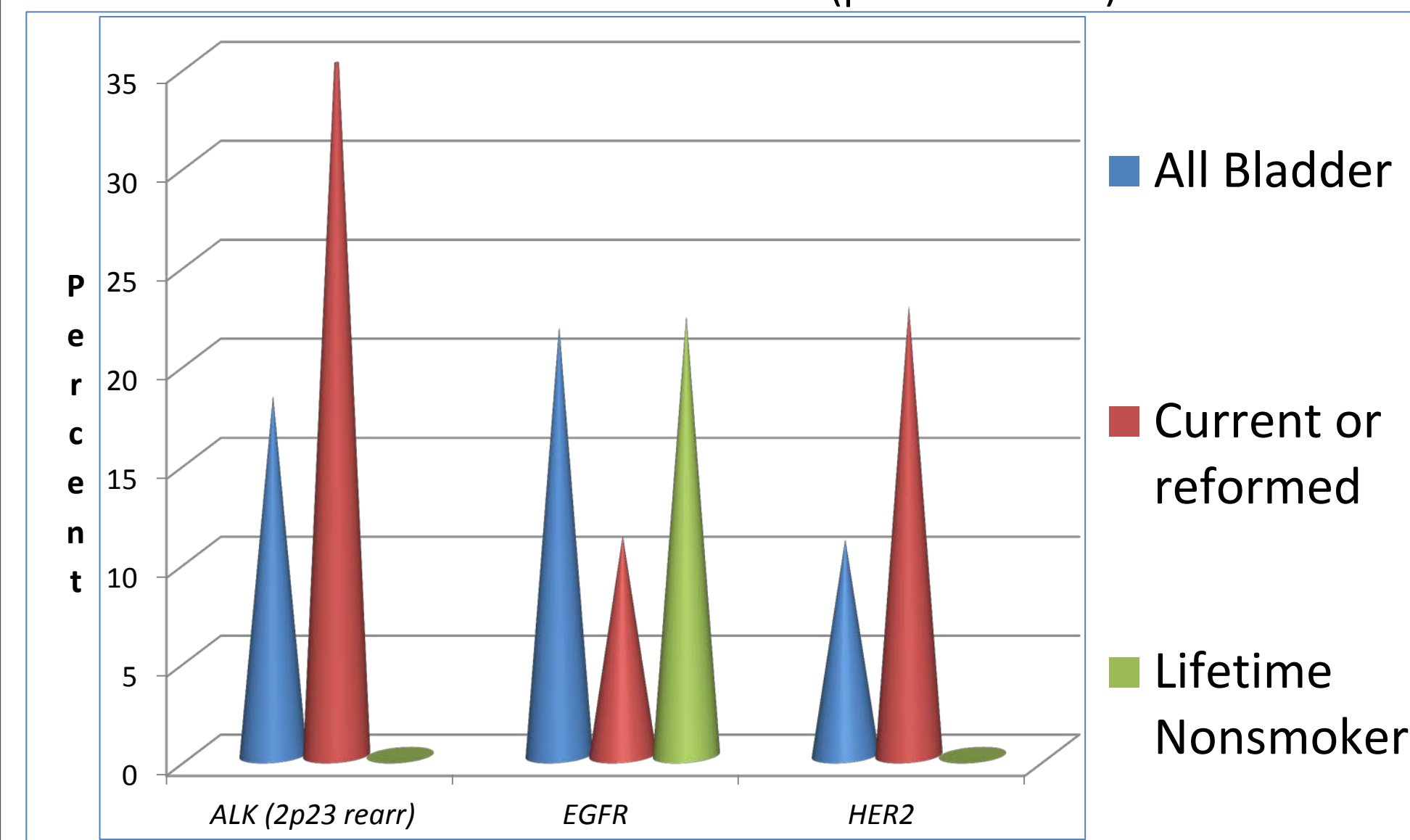
Results, Gene Sequencing

Figure 3. Mutation frequency. Genes are shown from left to right by decreasing percent mutation. Genes with no alterations identified included *ALK*, *CSF1R*, *FGFR1*, *FLT3*, *GNA11*, *GNAQ*, *GNAS*, *JAK2*, *JAK3*, *MPL*, *NOTCH1*, *NPM1*, *PTPN11*, *VHL*. Genes seen in just one case included *ABL*, *AKT1*, *ATM*, *BRAF*, *CDH1*, *cKIT*, *cMET*, *HNF1A*, *KDR*, *MLH1*, *NRAS*, *PDGFRA*, *RET*, *SMAD4*, *SMARCB1*. Additionally, while no cases in the subgroup were tested for *BRCA1/2*, the overall incidence in bladder cancer was 6.5% for *BRCA1* and 13% for *BRCA2*.



Results, Fluorescence or Chromogenic in situ Hybridization (ISH)

Figure 4. Amplification or rearrangement. *HER2* amplification was not seen in documented lifetime nonsmokers (p value = 0.05).



Results, Overall Survival

Figure 5. Kaplan Meier Curve. Overall survival curves of NS (red) versus R/S (blue) from the date of diagnosis. Statistical significance was not reached ($p=0.21$) due to small sample size.

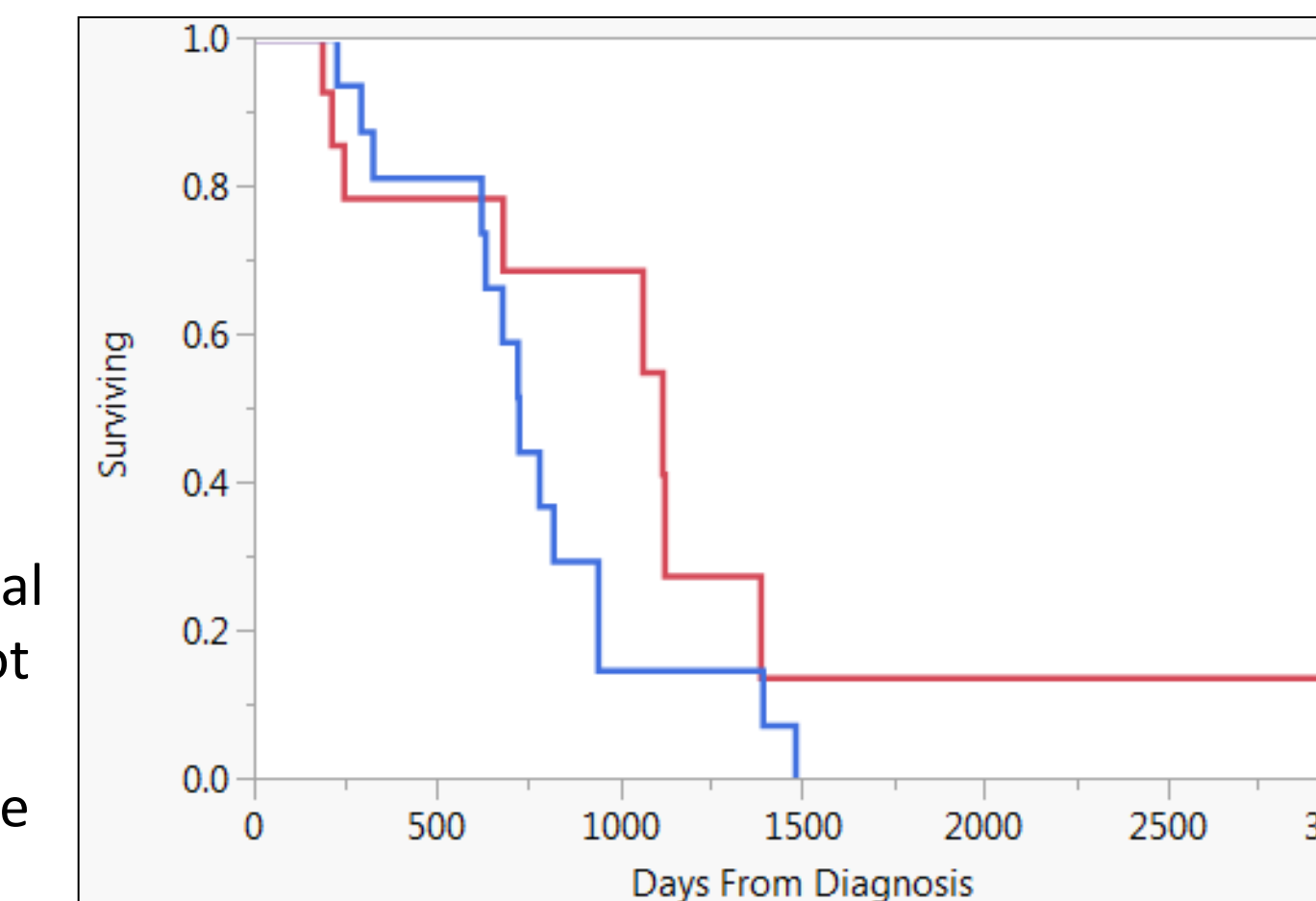


Table 2. Average survival, range, and vital status. Average survival was 175 days longer in the NS cohort.

	Avg Days Survived	Range	Alive
NS, n=14	843	185 – 2966	6/14
R/S, n=17	668	112 – 1479	3/17

Conclusions

- The difference in molecular biology between R/S and NS with BC suggests a different oncogenesis, with potentially different treatment options.
- More studies need to be conducted to identify other mutational abnormalities between smokers vs. lifetime nonsmokers.
- Increased incidence of *PIK3CA* mutations in NS may inform clinical trial design in this subgroup of BC patients.
- BRCA1* and *BRCA2* testing in bladder cancer might identify a subset for PARP inhibitor clinical trials.
- Follow-up on the *ALK* translocation patients is recommended to determine whether crizotinib was utilized and, if so, review associated outcomes.

References

- Cancer Genome Atlas Research N: Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* 507:315-22, 2014.
- Freedman ND, Silverman DT, Hollenbeck AR, et al: Association between smoking and risk of bladder cancer among men and women. *JAMA* 306:737-45, 2011.
- Millis et al. Molecular Profiling of Infiltrating Urothelial Carcinoma of Bladder and Nonbladder Origin. *Clinical Genitourinary Cancer*, 2014.